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Morphology and quantification of fungal growth in residential dust and carpets

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Abstract

Mold growth indoors is associated with negative human health effects, and this growth is limited by moisture availability. Dust deposited in carpet is an important source of human exposure due to potential elevated resuspension compared to hard floors. However, we need an improved understanding of fungal growth in dust and carpet to better estimate human exposure. The goal of this study was to compare fungal growth quantity and morphology in residential carpet under different environmental conditions, including equilibrium relative humidity (ERH) (50%, 85%, 90%, 95%, 100%), carpet fiber material (nylon, olefin, wool) and presence/absence of dust. We

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Author contributions

NN wrote manuscript, performed microscopy and qPCR analyses. SH provided carpet and house dust samples, along with fungal sequencing data. HS, AD, & MP performed Alt a 1 allergen analysis. LX, MB, CR, & BG aided with fungal morphology and microscopy techniques. KD designed the project, oversaw project, and contributed to manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

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analyzed incubated carpet and dust samples from three Ohio homes for total fungal DNA, fungal allergen Alt a 1, and fungal morphology. Dust presence and elevated ERH (≥85%) were the most important variables that increased fungal growth. Elevated ERH increased mean fungal DNA concentration ($P < 0.0001$), for instance by approximately 1000 times at 100% compared to 50% ERH after two weeks. Microscopy also revealed more fungal growth at higher ERH. Fungal concentrations were up to 100 times higher in samples containing house dust compared to no dust. For fiber type, olefin had the least total fungal growth, and nylon had the most total fungi and *A. alternata* growth in unaltered dust. Increased ERH conditions were associated with increased Alt a 1 allergen concentration. The results of this study demonstrate that ERH, presence/absence of house dust, and carpet fiber type influence fungal growth and allergen production in residential carpet, which has implications for human exposure.

Keywords

Microscopy; Mold; Indoor air quality; Flooring; Moisture; Fungi

1. Introduction

Asthma costs $81.9 billion per year in the United States alone [1] and affects 8% of the US population [2]. This disease disproportionally affects low-income communities and is associated with poor quality housing conditions [3]. Exposures within the residential built environment are important because 90% of our time is spent indoors [4], and these exposures may be responsible for up to 44% of physician-diagnosed asthma cases [5]. Common asthma triggers include mold products (e.g., antigens, inflammatory factors) that can contaminate building materials or deposit within house dust reservoirs.

Exposure to fungi in housing costs an estimated $22.4 billion per year, and losses include medical expenses, reduced work/school productivity, and lower quality of life [6]. Fungal exposure in the indoor environment is associated with an increased risk and severity of asthma and allergies [7–11]. Fungi contain many known allergens [12], including Alt a 1, a major fungal allergen found in the ubiquitous species *A. alternata* and related taxa within the Ascomycota order, Pleosporales [13]. Alt a 1 is a highly conserved allergen that can be found in Alternaria sp. and other common indoor fungal sources in the Pleosporales order, such as *Ulocladium* and *Stemphylium* [14–18]. *Alternaria* sp. and other cross-reactive fungal species that can produce the Alt a 1 allergen are present in 95–99% of homes [19,20]. It has known health effects, including being a risk factor for asthma and allergic rhinitis [21–25]. In addition to the inhalation of fungal components (spores, hyphae, and fragments), fungi can also release harmful chemicals, such as microbial volatile organic compounds (MVOCs) and mycotoxins into the air [26, 27]. In occupational settings, mycotoxins have been found to be associated with hypersensitivity pneumonitis, and with long term exposure, resulting in liver cancer [28]. MVOCs can lead to irritation of the eyes and upper respiratory tract as well as inflammatory reactions [29].

Fungal growth (indicated by visible mold or moldy odor) in a home is the strongest and most consistent feature associated with negative health effects [9,30]. Water availability is

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generally the limiting factor for growth indoors because nutrients are provided in sufficient quantity to sustain fungal growth on dust particles [31,32]. Moisture may result in conidia germination, that can result in the additional release of allergens, including Alt a 1 [33,34]. Additionally, even relative humidity levels in the air can be sufficient to support microbial growth indoors [31], and dust itself is hygroscopic [35,36]. Dust is typically present on floors in homes at a loading of 1–20 g/m^2 [37]. In the presence of excess moisture, fungi can grow on many substrates commonly found in residential homes including wood, insulating foam, wallpaper, concrete, and carpet [38]. Previous studies have shown that fungal growth is activated around elevated ERH conditions greater than 80% in building materials such as wallpaper and gypsum [39]. This is a growing area of concern as major storm events and increased stormwater runoff events due to urbanization are increasing the number of flooding events in residential homes [40].

Fungal growth indoors can include both unicellular and filamentous fungi. Filamentous fungi form long branching structures called hyphae that spread and form large networks called mycelia. Filamentous fungi, such as species placed in the order Eurotiales, form mycelium networks that include aerial hyphae that produce conidiophores from which emerge conidia. Abiotic or biotic disturbances can result in aerosolization and dissemination of these fungal bioaerosols, such as fungal fragments and especially spores/conidia that can be released into the air [41,42]. The optimal growth conditions for fungi vary by taxa, but most require typical ambient temperatures (such as 25 °C) and a water activity (a_w) value of at least 0.65 [41]. Increased growth generally occurs at higher a_w levels [43].

Fungal growth on building materials such as drywall and wood have been well-studied [39,44]. The type of building materials within the indoor environment play a crucial role in the types of microorganisms present and their ability to grow [45,46]. One material of particular interest is carpet. Resuspension of particles containing microbes following a disturbance on carpets is an important source of human exposure [47]. According to the Carpet and Rug Institute, carpets are the most common flooring material used in the built environment accounting for 51% of the total U.S. flooring market [48]. Carpet fibers can retain moisture, and the relative humidity in a carpet may be elevated above the surrounding room air [49]. Nylon carpet fibers (nylon 66 and nylon 6) have made up the majority of sales for residential carpets for decades [50,51]. Currently, there is a paucity of information in the peer-reviewed literature regarding fungal growth within carpets and dust as well as the role of factors such as carpet type, dust availability, and how relative humidity influences growth. We need to understand how conidia/spore attachment and hyphal growth occurs in varying indoor environmental conditions to provide insights for people afflicted with respiratory diseases. This knowledge is necessary to improve recommendations for cleaning practices, carpet material design and selection, and optimal indoor conditions. Ideally, this information can eventually lead to improvements in building design to support healthy microbial communities [52,53].

This study aims to characterize growth of fungi within carpets and identify differences in growth with varying (1) ERH levels, (2) fiber materials, and (3) presence or absence of house dust. We hypothesized that the presence of house dust, higher ERH conditions, and natural fibers such as wool would stimulate increased fungal growth in carpet in comparison

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to no dust, lower ERH, and synthetic fibers. As a second indicator of fungal colonization, we also specifically consider the presence and production of the major fungal allergen Alt a 1 at each ERH condition.

2. Materials and methods

2.1. Experimental overview

Separate experiments were conducted which included an ERH Experiment (carpet with dust and dust only) (Fig. 1A) and a Materials and Dust Loading Experiment (Fig. 1B). These experiments were intended to test the effect of ERH, carpet fiber type, and dust loading on fungal growth on carpets. The materials and dust loading experiments were conducted together using the same samples in an experimental matrix. Total fungal DNA was quantified for all samples. *A. alternata* quantities were quantified for samples inoculated with *A. alternata* in the materials and dust loading experiments. The Alt a 1 protein was also quantified for all ERH conditions and the original dust (before incubation) using "Fiber-Dust" samples.

2.2. Carpet samples

Carpets used for the ERH treatment study were collected from three residential homes in Ohio beginning in May of 2016 using a previously described sampling protocol [31] (Table S1). Household dust used in this study was collected from the same residents' vacuum cleaners and filtered through a 300 μm sieve to remove large particulates. Carpet samples were stored in airtight plastic bags at room temperature until used in this study. Sieved dust samples were stored at room temperature in glass beakers covered with parafilm until use. New carpet was purchased which included 100% nylon, olefin (94% polypropylene, 6% nylon), and 100% wool carpet fibers and contained no antimicrobial coatings for use in this study. Carpet information for each sample including pile height, total mass of fibers, and material types were recorded (Table 1).

All carpet samples were cut into 5 cm × 5 cm squares and autoclaved at 121 °C for 1 h and dried at 100 °C overnight (~12 h) prior to incubation. Site 1 house dust for dust loading and fiber material experiments were also autoclaved in this way for the samples labeled as "Autoclaved House Dust" conditions. Carpet samples and house dust samples autoclaved in this manner did not exhibit growth when placed on a potato dextrose agar plate indicating a high degree of sterilization. However, it is unlikely that we achieved complete sterilization for these sample types as described in previous literature [54]. In carpet samples containing the household dust, a modified ASTM method F608-13 with a 12 cm long, 1440 g steel pipe was used to embed 50 mg of household dust into each carpet square from the same home avoiding a 1 cm area bordering the edge of the sample [31]. The dust loading quantity in our samples translates to 20 g of house dust per square meter of carpet which is consistent with previously observed loading values on flooring surfaces [37].

This study was approved by the Institutional Review Board at The Ohio State University (study 2016B0132).

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2.3. Fungal strain

A freeze-dried *A. alternata* strain was purchased from ATCC (Manassas, VA, USA), item number 66981, and rehydrated in sterilized distilled H2O overnight (~12 h). The rehydrated fungal strains were vortexed for 15 s and 10 μL aliquots were placed onto Potato Dextrose Agar (PDA) [Difco Potato Dextrose 24 g; Agar 15 g; Distilled H_2O 1 L] culture plates. The PDA plates were incubated for 2 weeks at 25 °C. Media was supplemented with 0.025 g of chloramphenicol (Sigma Aldrich, St. Louis, MO, USA) to prevent bacterial contamination. *A. alternata* spores were harvested using a modified plate flooding method in which PBS-T [PBS + 0.1% Tween-20] was poured into each PDA plate, scraped with an inoculating loop, and the spore charge was then poured into a flask containing 2 mm garnet beads (ASTM G26). This solution was shaken vigorously to release spores from the hyphae and then filtered through sterile wool. This process was repeated to obtain a 10^6 spores/μL solution. Spores were resolved and counted by staining [Crystal Violet (Sigma Aldrich, St. Louis, MO, USA) 10 μL; Tween-20 (Fisher Bioreagents, Waltham, MA, USA) 10 μL; Spore Solution 10 μL; Distilled H_2O 970.μL], 10 μL of which was aliquoted onto a 3 separate InCyto DHC-N01-5 Neubauer Improved C-Chips and viewed with a Labomed microscope with a 20x air objective lens. Approximately 11% of the fungal particles in the *A. alternata* spore suspension were fragmented hyphae as determined by microscopy.

2.4. Relative humidity control

Salt solutions were used to control ERH conditions inside of the incubation chambers and were comprised of $MgCl_2$ and NaCl. For 50% ERH, 44.84 g of $MgCl_2$ was added to 100 mL of DI water [31]. For 85, 90, and 95% ERH, a total of 46.76, 35.89, and 27.54 g of NaCl was added to 100 mL of DI, respectively. The water activity of each salt solution was measured on an Aqualab 4 TE Dew Point Water Activity Meter (Decagon Devices, Pullman, WA, USA) and adjusted if needed. The water activity measured in each salt solutions was in equilibrium with the ERH in each incubation chamber. The highest tested ERH (100%) was achieved by using deionized water (DI) only. A single layer of parafilm was placed over the top of each 3.8 L incubation jar to retain moisture inside and allow for CO_2 transfer. Onset® HOBO® loggers (Bourne, MA USA) were placed in the incubation chambers to confirm ERH conditions stayed constant during the incubation period.

2.5. Inoculation and incubation

For samples inoculated with *A. alternata*, spores were deposited onto carpet squares using a Medline Aeromist Compact Nebulizer compression kit. The *A. alternata* spore solution was diluted in PBS to a 10^6 spores/mL concentration. The diluted spore solution (3 mL) was placed into the nebulizer tank. The 5 cm × 5 cm carpet squares were placed, fiber side up, into a 3.8L glass jar. A 5/16″ hole was drilled into the glass jar's aluminum lid. Flexible plastic tubing was attached to the nebulizer tank and fed through the newly drilled hole in the aluminum lid (Fig. 2). The compressor was turned on for 10 min at a nebulizer fluid consumption rate of 0.18 mL/min to release the spores into the 1.9 L chamber. The aerosol particles were then allowed to settle in the chamber for an additional 10 min before placing the carpet samples into their incubation chambers. To demonstrate the spore coverage achieved on each carpet coupon, the inoculation process was repeated using crystal violet

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dye. The dye showed that the spore mist was able to reach every area of the carpet coupon and an estimated 290,000 spores fell on each carpet sample (spore deposition calculated using concentration of spore solution and area of carpet coupon). Actual spore quantity deposited on carpet may be lower due to the assumption that all spores fell evenly to the bottom of the incubation chamber and that no spores exited the chamber during inoculation.

All samples were incubated in sterilized 3.8 L glass jars (Fig. S1) at 25 °C for 2 weeks in a VWR incubator (Radnor, PA 19087). Carpet samples from each site were placed into a common chamber and each separated by previously baked (550 °C) tinfoil to prevent any cross-contamination of the samples by contact. The tinfoil was arranged such that no excess moisture build up would occur near the samples. A salt solution (100 mL) was placed inside the beaker in each chamber to create the ERH condition being tested. Incubation chambers were checked multiple times per day and parafilm changed daily to prevent loss of ERH and prevent CO_2 accumulation. Generally, the ERH remained at ± 2.5% of the desired ERH condition.

2.6. Microscopy

After 2 weeks of incubation, carpet fibers were cut from the sample squares using aseptic techniques and approximately 1.25 mg placed on Fisher Scientific (Hampton, NH USA) glass microscope slides (25 × 75 × 1 mm). For confocal and light microscopy, samples were fixed in 4% Paraformaldehyde (PFA) Solution in PBS (Affymetrix, Santa Clara, CA, USA). PFA solution (100 μL) was placed on each sample and allowed to sit for 2 h. Uvitex2B (50 μL) was applied directly to the fixed samples and allowed to sit for 5 min. PBS was gently applied to the samples to rinse and was carefully pipetted off to prevent removing fungal structures. All samples were stored in the dark until microscopic analysis was performed on that same day. Uvitex 2B is a fluorescent stain (excitation 385 nm, emission 480 nm) for fungal polysaccharides that is effective for highlighting fungal structures (e.g., chitin) [55]. Sample preparation for Scanning Electron Microscopy (SEM) imaging consisted of extracting fibers and placing them on an aluminum stud with double-sided black carbon tape. The samples were then sputtered with 10 nm of gold to dissipate heat from the focused electron beam.

Fluorescent microscopy analysis was performed on a Nikon A1R Inverted Confocal (Nikon Insttruments Inc. Melville, NY USA) at the Campus Microscopy and Imaging Facility (Ohio State University). SEM imaging was performed on an Apreo LoVac Scanning Electron Microscope at the Center for Electron Microscopy and Analysis (Ohio State University).

2.7. Quantification by qPCR

Quantitative polymerase chain reaction (qPCR) was used to quantify total fungal DNA quantity in all samples as well as quantify *A. alternata* species in dust loading and materials experiment samples. qPCR was conducted in triplicate on extract from one physical sample for each condition. We took the average value of the three sites for analysis and also for calculation of variation in the ERH experiment samples (Fig. S2). Values were converted to “spore equivalent” units based on a standard curve of known *A. fumigatus* or *A. alternaria* spores using six 10-fold serial dilutions. This value was divided by the amount of material in

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which the DNA was extracted. DNA was extracted from 50 mg of "Fiber-Dust" (ERH, carpet fiber, and dust loading samples) and house dust (ERH samples only). In this analysis, fiber-dust refers to the combination of carpet fiber material, embedded house dust, and biomass grown during incubation which was extracted by cutting the fibers from the original 5 cm ÷ 5 cm carpet sample. Vacuumed dust samples were collected using a Eureka! Mighty Mite Vacuum cleaner with a modified attachment that held a 1.2 μm MCE filter (SKC, Eighty Four, PA, USA) in which the dust was collected.

DNA was extracted using a modified DNeasy Powerlyzer Power Soil Kit (Qiagen, Hilden, Germany) using a bead mixture (1 g 2 mm garnet beads, 0.1 g 0.5 mm glass beads, and 0.3 g 0.1 mm glass beads) to efficiently break up fungal cell walls [56]. DNA was extracted from each carpet square and then analyzed in triplicate utilizing the developed qPCR (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA USA). ERH samples were diluted to 100X to prevent inhibition and materials/dust loading samples were diluted to 10X for qPCR analysis. qPCR was performed on an Applied Biosystems Quantstudio 6 Flex (Fisher Scientific, Waltham, MA, USA) and analyzed using Quantstudio Real-Time PCR Software v1.2 with a total reaction volume of 25 μL with SYBR Green PCR master mix.

Total fungal qPCR was determined using a fungal forward-primer FF2 (5′-GTTAAAAAGCTCGTAGTTGAAC-3′) and reverse primer FR1 (5′-CTCTCAATCTGCAATCCTTATT-3′) as a "universal" fungal primer derived from the fungal 18 S rRNA gene [57]. qPCR conditions for FF2/FR1 reactions included 1 cycle of 50 °C for 2 min and 95 °C for 10 min followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. "Universal" fungal qPCR reactions included 12.5 μL of Applied Biosystems SYBR™ Green PCR Master mix (Fisher Scientific, Hampton, NH, USA), 0.75 μL 10 μM FF2 primer, 0.75 μL 10 μM FR1 primer, 9 μL DNAse free water (Thermo Fisher Scientific, Waltham, MA, USA), and 2 μL of extracted sample DNA per well. *A. alternata* primers were used for species specific quantification in all samples. These primers were designed based on alignment of β-tubulin gene sequence, forward-primer AaltFor (5′-GTGCCTTCCCCAAGGTCTCCG-3′) and a reverse-primer AaltRev (5′-CGGAAACGAGGTGGTTCAGGTC-3′) [58]. Temperature-time profile for AaltFor/AaltRev primers included 1 cycle of 50 °C for 2 min and 95 °C for 10 min, followed by 40 cycles of 95 °C for 30 s and 70 °C for 1 min. *A. alternata*-specific qPCR reactions included 12.5 μL of Applied Biosystems SYBR™ Green PCR Master mix (Fisher Scientific, Hampton, NH, USA), 0.125 μL 100 μM AaltFor primer, 0.125 μL 100 μM AaltRev primer, 10.25 μL DNAse free water (Thermo Fisher Scientific, Waltham, MA, USA), and 2 μL of extracted sample DNA per well.

Before qPCR analysis, DNA extraction efficiencies were determined for each fiber material by spiking 20 μL of a 10^6 spores/μL solution of *A. fumigatus* onto 50 mg of each fiber material. DNA extraction efficiencies for nylon, olefin, and wool fiber were calculated using Equation (1) and deviations were determined by using propagation of error (Equation (2)). Nylon and wool fiber materials showed nearly 100% extraction efficiency of spike DNA compared to the amount recovered from the spike with no carpet fibers present, which was used for our standards. The DNA extraction method was not as efficient with olefin fibers which only showed 56% efficiency, potentially due to inhibition of the fiber material (Table

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S2). These extraction efficiencies were used only in fiber-dust qPCR analyses. qPCR values for each sample were calculated to units of spore equivalents per mg of fiber-dust (Equation (3)). qPCR inhibition tests were performed on all samples by using a *A. fumigatus* spore extract as a spike and no inhibition of the reaction was detected (data not shown).

$$Efficiency\ (\%) = \frac{Quantity\ from\ fiber\ material}{Quantity\ from\ spore\ solution} * 100 \quad (1)$$

$$\sigma = \sqrt{\left(\sigma_{spore\ solution}\right)^2 + \left(\sigma_{fiber}\right)^2} \quad (2)$$

$$\frac{Spore\ Equivalents}{mg\ of\ Fiber-Dust} = \frac{Spore\ Equivalents}{\mu\ L\ of\ DNA\ Extract} \times \frac{50\ \mu\ L\ DNA\ Extract}{50\ mg\ of\ \text{“}Fiber-Dust\text{”}} \quad (3)$$

2.8. Fungal sequencing

Total DNA extracted from dust samples (Site 1, 2, 3) was sequenced on Illumina MiSeq at RTL Genomics in Lubbock, TX. This sequencing used fungal rRNA amplicons (ITS1F and ITS2aR ribosomal DNA primers [59]) with 2 × 300 bp sequencing reads. Data (raw fastq files) was analyzed with the bioinformatics pipeline QIIME (version 1.9) [60]. The spacers and primers were trimmed and the paired end reads were joined utilizing the SeqPrep method [61]. Sequences were trimmed to a Phred score of 20 with 3 low-quality base cells allowed before truncating.

Fungal taxonomy represented in the samples was determined using BLAST version 2.2.28+ [62] along with the UNITE database [63] and FHiTINGS version 1. 4 [64]. Absolute abundance of organisms in the samples was determined by dividing the number of sequences from each species by the total number of sequences in each sample. This proportional value was then multiplied by the spore equivalents of total DNA as measured by qPCR [65].

2.9. Alt a 1 allergen measurement

Alt a 1 was chosen as a model allergen for this study due to its presence in a majority of homes and the potential negative health impacts on residents. Carpet “Fiber-Dust” samples were extracted with PBS 0.05% Tween, pH 7.4 (Sigma Life Science, St. Louis, MO, Cat #P3563-10PAK) at a concentration of 100 mg/mL for 1 h at 30 °C using a rotating shaker. These samples were stored at −20 °C until analysis. Alt a 1 was measured by ELISA (Indoor Biotechnologies, Charlottesville, VA, Cat # EL-AA1) [13] with amplification using a streptavidin poly-HRP80 conjugate (Fitzgerald Industries International, North Acton, MA, Cat # 65R–S105PHRP) [66]. This enhanced the sensitivity of the assay ranged from the published 200 pg/mL to ~4 pg/mL (data not shown). Along with the standards and samples, a diluted allergen extract of an *A. alternata/Alternaria tenuis* (Stallergenes Greer, Boston, MA, Cat # XPM1D3A25) was run as a quality control to confirm that the amplified assay was not over- or under-estimating results.

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2.10. Statistical analysis

All samples analyzed by qPCR were done in triplicate and the results were analyzed with Statistical Analysis Software (SAS), version 6.2.9200. Statistical significance was considered $P < 0.05$. We tested the hypothesis that increased ERH would be associated with increased fungal DNA concentration by comparing the amount of fungal DNA present in different ERH conditions for each site using a Spearman Rank Correlation Coefficient. We also tested the hypothesis that differences in fiber material and dust loading would be associated with differences in fungal DNA concentration. Differences in the amount of fungal DNA present in different fiber materials and dust loading samples were compared using a Satterthwaite Two Sample *t*-test and Pearson Correlation Coefficient. Statistical analyses on Alt a 1 concentration included Satterthwaite Two Sample *t*-test and Spearmen Rank Correlation Coefficient.

3. Results

Microscopic evaluation revealed fungal structures on carpet fibers, including fungal spores/conidia (Figs. 3, 5D and 8) and hyphae (Figs. 3, 4C–E, 5B–E, 7, and 8B). Spore/conidia chains (Fig. 3A), septate hyphae (Fig. 3A and B), and conidiophores (Figs. 3B, 4C and 5D, and 8B) were also observed indicating growth and reproduction of fungi within the carpet materials (Fig. 3). Additional SEM examples of fungal growth on carpet fibers can be found in supplemental information (Fig. S3). qPCR analyses showed differences in ERH conditions (Fig. 6), dust loading (Fig. 9), and type of carpet fiber materials (Fig. 9) affected fungal quantities and Alt a 1 production (Fig. 10). Results are detailed below.

3.1. Relative humidity

We evaluated differences in fungal morphology and quantity in carpet at different ERH levels. 50% ERH showed few fungal spores and no growth for all sample sites (Figs. 4A and 5A). Qualitative microscopic observations revealed fungal spore quantity slightly increased at 85% ERH (Figs. 4B and 5B). At 90% ERH hyphae were observed on carpet fibers indicating fungal growth (Figs. 4C and 5C). At 95 and 100% ERH similar fungal quantities were observed (Fig. 4D–E and 5D–E), however, the presence of conidiophores and conidia chains was qualitatively greater at 95% ERH (Fig. 5D). No fungal structures were observed via SEM imaging in site 3 samples at 90, 95, or 100% ERH conditions. This may have been due to the limited sensitivity of SEM compared to the full-size of the carpet coupon as qPCR results showed substantial fungal colonization of these samples (Fig. 6). Microscopy revealed that fungal hyphal networks directly on the carpet fibers increased in size and numbers with increasing ERH (Fig. 4).

SEM imaging showed a similar trend as the fluorescence microscopy, with little to no fungal growth at 50% and 85% (Fig. 5A–B), small hyphal structures began to emerge at 90% ERH (Fig. 5C), and elongated hyphae with conidia production at 95% and 100% ERH (Fig. 5D–E). The majority of fungal conidia shown in SEM images (Figs. 5, 8 and 11) were consistent as *A. sydowii* due to their globose to sub-globose morphology characterized by a spiny outer cell wall surface ornamentation. This species was also highly abundant in the sample based on Illumina sequencing of the ITS region at 100% ERH (Table S3). For each site location

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the original dust (before incubation) was also sequenced (Table S4) and revealed the preexisting presence of *A. sydowii* Fig. 5 shows an example of carpet samples at low to high ERH conditions viewed on SEM.

For ERH samples, total fungal DNA quantities were measured using three different measurements as the normalizing factor including (1) fiber-dust, a combination of fibers and dust, (2) dust incubated without carpet, and (3) dust only, that was extracted from carpet with a Eureka! Mighty Mite vacuum after incubation. As ERH increased, the total fungal concentration also generally increased. The total fungal quantity from Sites 2 and 3 increased with increasing ERH. Site 1 showed similar trends from 50% to 95% ERH but the total quantity at 100% ERH was lower than that at 95% ERH. For each site, the total fungal concentration normalized to fiber-dust mass were significantly lower at all ERH conditions when compared with dust only and vacuumed dust sampling methods (Fig. 6). We calculated associations between fungal concentrations and ERH levels in dust using a Spearman Rank Correlation Coefficient (Table 2), and did this comparison with each sampling method ("Fiber-Dust", dust only, and vacuumed dust). We used the average fungal concentration value as determined by qPCR for each of the 3 sites at all 5 ERH conditions (15 points total for each sampling method) for the Spearman Rank calculations. Fungal DNA quantity for each site and sampling method exhibited strong correlations ($r_s > 0.86$) and statistical significance ($P < 0.0001$) with ERH level.

3.2. Carpet fiber material and dust loading

The effects of carpet fiber materials and dust loading on fungal growth was determined by inoculating *A. alternata* onto carpet samples and incubating at 100% ERH. A total of three carpet fiber materials were tested that included olefin, wool, and nylon using newly purchased carpet. Three different dust loading scenarios were also tested with each carpet fiber material: carpet containing no dust, carpet containing autoclaved house dust, and carpet containing unaltered house dust. All dust originated from Site 1.

SEM analyses of *A. alternata*-inoculated carpet fiber materials showed that fungal growth appeared most abundant in olefin fibers with high quantities of conidia and large hyphal networks (Fig. 7C). Wool exhibited the second highest fungal growth with moderate to large hyphal structures and conidial chains (Fig. 7B). Nylon showed the least amount of fungal growth with minimal presence of conidia and small hyphae (Fig. 7A).

Through SEM analysis, qualitative observations showed that *A. alternata*-inoculated samples containing no dust had almost no observable fungal structures. In autoclaved dust, we observed hyphal structures consistent with *A. alternata* hyphae in low quantities, but spores were not found (Fig. 7C). No *A. alternata* was definitively observed in house dust loaded samples. Other fungal species, putatively identified as *Aspergillus* and *Penicillium* spp., were observed in both autoclaved and house dust loaded samples, with larger quantities in the latter. Fungal growth was observed in house dust in all experiments (Fig. 8).

Total fungal and *A. alternata* quantities were determined via qPCR for dust loading and carpet fiber material experiments (Fig. 9). A qPCR comparison of fungal DNA quantities estimated by total fungal primers (FF2/FR1) and *A. alternata* specific primers (AaltFor/

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AaltRev) was done by quantifying the *A. alternata* standards used as samples using total fungal primers with *A. fumigatus* standards. This comparison showed that *A. alternata* DNA quantities should be increased by a factor of 2, when comparing total fungal load to the *A. alternata* specific load. This is due to the different region of the fungal DNA amplified by each primer set, with the multiple-copy ITS region for FF2/FR1 and single-copy β-tubulin for AaltFor/AaltRev.

Olefin fibers showed the lowest amount of total fungi in all dust loading conditions. Wool fibers showed the largest amount of total fungal quantities in no dust and autoclaved house dust samples, while nylon fibers showed the greatest in unaltered (non-sterilized) house dust. For sterilized carpet samples with *A. alternata*, wool fibers with autoclaved house dust showed the greatest increase in fungal growth, followed by nylon then olefin fibers. In unaltered house dust, nylon fibers showed the greatest *A. alternata* quantity followed by olefin then wool fibers. All fiber materials showed little to no growth in samples containing no dust.

Samples containing no dust showed almost no fungal growth in both total fungal and *A. alternata* measurements. Total fungal quantities were highest in samples containing unaltered house dust, while these quantities in autoclaved house dust were only slightly elevated compared to samples containing no dust. Inoculated *A. alternata* quantities were highest in samples containing autoclaved house dust, while only small quantities were measured in both samples with no dust and unaltered house dust (Fig. 9). We found that both material type and dust loading were statistically significantly associated with differences in fungal concentration in carpet ($P < 0.05$) with the exception of total fungal load of nylon and olefin fibers with no dust as well as *A. alternata* loads in nylon and olefin fibers with no dust and wool and olefin fibers with house dust.

3.3. Alt a 1 allergens

Alt a 1 was quantified in original dust samples (no incubation) and in all ERH treatment conditions (50, 85, 90, 95, and 100% RH) after 2 weeks for all three site locations using the “Fiber-Dust” sampling method. Site 1 and 2 Alt a 1 allergen quantities were very similar in the original dust and throughout all ERH conditions (Fig. S4). Site 3 original dust contained approximately 10 times more Alt a 1 when compared to Sites 1 and 2. Once incubated with increasing humidity, similar trends were observed in all three sites with increasing Alt a 1 allergen quantities starting at 95% ERH (Fig. 10). For all sites, Alt a 1 showed minimal to no increase in concentration for 50, 85, and 90% ERH, when compared to each site’s respective original dust concentration (Fig. S4). At 95% ERH, Alt a 1 allergen quantity increased by 10 times the original dust, while at 100% ERH this increased to 100 times the original dust for all three sites (Fig. S4). Alt a 1 allergen concentrations for all three sites were averaged and similar trends were observed for the combined data (Fig. 10). Alt a 1 allergen concentration exhibited a strong correlation ($r_s > 0.86$) and statistical significance ($P < 0.0001$) with ERH level. Satterthwaite two sample t-tests also showed statistical significance ($P < 0.05$) differences in Alt a 1 concentration when comparing the original dust and 95%–100% ERH conditions (Table S6).

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4. Discussion

ERH conditions, carpet fiber material, and presence of house dust all have significant impact on fungal growth in carpet. Elevated ERH conditions of greater than 90% and the presence of house dust favored increased fungal growth as indicated by fungal DNA abundance and visualization of fungal vegetative (hyphae) and reproductive (conidiophores and conidia) structures. Fungal growth in carpet fiber material was varied depending on the dust loading type. Wool fibers showed the most fungal growth in no dust and autoclaved house dust conditions, while nylon fibers showed the most in unaltered house dust. In all dust loading conditions, olefin fibers facilitated the least amount of fungal growth. These results align with previous quantitative results of fungal growth in house dust [31] and reiterates the strong link between fungal growth and moisture. Elevated ERH (>90%) also favored the production of the Alt a 1 allergen. The presence of these fungal structures reiterates that fungi can grow if sufficient moisture is available.

If sufficient moisture is present, the second most important factor to promote fungal growth was the presence of house dust to promote fungal growth in carpet. Quantitative analyses showed a significantly lower quantity of fungal growth on carpet samples containing no house dust compared with autoclaved and unaltered house dust. This was further demonstrated by increased fungal quantities in dust even in the absence of carpet fibers. The fungal DNA abundance was substantiated by microscopic (fluorescence and scanning electron microscopy) visualization of fungal structures on dust and carpet fibers compared to samples lacking any dust. House dust can be an important source of nutrients such as organic carbon, nitrate, phosphate, and sulfate providing levels 4 times greater than the stoichiometric requirements for microbial growth [31]. Fungal species, such as *A. versicolor* and *A. fumigatus*, can grow on many inorganic materials, especially in hygroscopic conditions and in the presence of absorbed dust that serves as a suitable substrate [67,68]. The combined presence of dust and moisture results in microbial growth and metabolic activity [69]. Fungal communities can even degrade chemicals found in the dust [70]. House dust is also highly variable in size and chemical contents based on geography, occupancy, presence of pets, ventilation, cleaning practices, and seasons which can all affect the quantity and diversity of microbial communities [71–73]. This highlights a need for better understanding of house dust chemistry and its influence on microbial growth. When compared to smooth flooring materials, carpets retain more dust [74] and also have higher particle resuspension rates [75,76]. In addition, standard vacuum cleaning usually only removes dust from the top layer of the carpet, allowing dust to collect in the bottom portion and remaining available for microbial uptake [77]. Studies have also shown that vacuum filter penetration can be ~10–37% for particles 0.5–3.3 μm, suggesting smaller fungal spores and fragments can potentially be resuspended into the air during the vacuum cleaning process [78]. Based on the similar fungal DNA quantities observed with dust only (no carpet) and vacuumed dust (from carpet), it may show that the synthetic carpet fibers do not contribute to fungal growth but act as a reservoir for the dust to settle and remain in the home. This highlights the importance of adequate dust removal in carpet to minimize potential fungal growth in flooring.

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Fungal DNA quantity generally increased as ERH conditions elevated. The presence of conidiophores and conidia in carpet samples with dust and at elevated ERH (≥80%), suggests these conditions create the significant risk of direct inhalational exposure to fungal conidia/spores, some of which can have negative impacts on human health. The conidia that were attached to carpet fibers did not appear to have any physical attachment method, but more likely were held in place by electrostatic charge or similar forces (Fig. 11) [79]. These spores are vulnerable to release in the air by abiotic and biotic disturbances such as walking across the carpet, air movement from fans/ventilation systems, and vacuuming. Our data suggest that to mitigate fungal growth and possible adverse health effects, occupants should consider monitoring their relative humidity for elevated levels. Currently, the US EPA recommends the relative humidity to be maintained between 30 and 50% and definitely below 60% [80]. Relative humidity is the measure of moisture content in the air which can be easily measured with a variety of instruments available at local hardware stores. ERH, as measured in the study, refers to the condition when the relative humidity in the air is in equilibrium with the water activity of the surface (in this case carpet) in an enclosed space [81]. It is important to note that elevated RH in the air is enough to saturate carpet fiber materials, including nylon, wool, and polypropylene, due to their hygroscopic properties [49]. Several of the ERH levels used in this study are higher than what may be experienced in the built environment. However, these levels are not unreasonable in a bathroom after a shower, next to a water leak, in a home with recent flooding, or other suboptimal condition that results in the infiltration of moisture.

We observed differences in fungal growth based on carpet fiber materials. Wool is a fibrous keratin protein, which can be a source of metabolizable amino acids. Several strains of fungi isolated from soils, including *Trichophyton* sp., *Fusarium* sp., *Trichoderma* sp., and *Cladosporium* sp., have been previously shown to metabolize wool fiber substrates utilizing kertinase enzymes to cleave di-sulfur bonds [82]. It is possible that other carpet-colonizing species may also have keratin degrading ability, such as Dermatophyte species and some plant pathogens such as *A. alternata* that has been shown to grow on wool carpet fibers containing no dust (Fig. 7B). *A. alternata* was observed tunneling through wool fibers indicating potential trophic interactions (Fig. 12). These observations were made in carpets with autoclaved dust so further investigations are needed to determine if keratin degradation was occurring in the samples.

In the presence of unaltered house dust, nylon fiber materials showed the highest total fungal and *A. alternata* quantity. The reasons for this finding could be investigated in future work. The samples containing no house dust showed the largest total fungal and *A. alternata* growth in wool fiber materials. This may be more indicative of the effect of carpet fiber material on fungal growth in the absence of house dust. In all cases, olefin carpet fibers showed the least amount of fungal quantities. This information can provide guidance for consumers, especially those sensitive to allergies or with asthma, for purchasing carpet materials, such as olefin or polypropylene blend, that may reduce their risk for harmful exposure to fungi and their metabolites. However, regardless of material type, controlling dust and moisture remain the most important factors.

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Alternaria sp. and their production of Alt a 1 can have significant impacts on respiratory health for sensitized populations [25,83,84]. Fungal sequencing data (Tables S3 and S4) and Alt a 1 allergen concentrations (Fig. 10) in this study also show that an increase in total *Alternaria* sp. present in "Fiber-Dust" samples corresponded to an increase in total Alt a 1 allergen concentration at 100% ERH. Site 3 samples contained a much higher baseline concentration of *Alternaria* sp. and 100X more Alt a 1 when compared to sites 1 and 2 (Table S7). Alt a 1 concentration for all sites increased with greater fungal growth as ERH increased and this increase was more prominent at highly elevated ERH (>95%) conditions. In addition to supporting more *Alternaria* growth, elevated ERH conditions have been shown to increase gene expression in fungal species [32] and spore/conidia germination, which could also increase Alt a 1 production in these conditions [85]. However, others have found that *A. alternata* spore counts, a major source of the Alt a 1 allergen, did not correlate well with Alt a 1 concentrations, suggesting the absolute amount of fungi is not the only determining factor for Alt a 1 levels [86]. This study did not determine if the increase in Alt a 1 concentration was a result of larger quantities of *Alternaria* sp. or that these species were producing more Alt a 1 at elevated ERH and future studies are needed to address this question.

Previous studies have also examined levels of fungi based on various building materials. Regarding carpets, Li and Kendrick showed that fungal quantities in the air are significantly higher in rooms that contain carpets compared to those without [87]. Nielson et al. showed increased fungal growth, measured as coverage (%), with elevated RH in a variety of residential building materials such as woods, plywood, gypsum, and wallpapers [39]. Similar studies have described fungal growth, as a frequency (%), on materials such as plaster, paint, concrete, and glass fibers from water damaged buildings [88]. Consistent with our results on the importance of ERH, studies involving different gypsum materials showed the effect of wetted conditions significantly altered the fungal community structure and metabolic activity compared to dry conditions [44]. Future studies on metabolic activity and better determination of fungal community compositions on carpet materials over varying ERH conditions could help elucidate when harmful fungal species begin to thrive.

Microbiologists and architects have already recognized the potential of utilizing the microbiology of the built environment to address real world health and sustainability concerns. Previously, the goal was to attempt to eliminate as many microorganisms as possible to create a "healthy" indoor environment. However, this goal is changing as we begin to understand that a lack of microbial diversity can cause immune system dysregulation, increasing occurrences of chronic diseases such as asthma and allergic rhinitis [89]. Defining and promoting a healthy biodiverse microbiome indoors, where we spend most of our time could improve health for building occupants [90]. We already know that we are engineering an indoor microbial ecosystem by building design choices such as architectural design, ventilation, and surface material types [91–93]. An improved understanding of fungal composition of different carpet microbiomes and the factors contributing to fungal growth in materials such as carpet can eventually lead to improvements in building design to promote healthy indoor microbial communities.

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4.1. Limitations

One limitation of this study is the small number of replicates for each experimental condition, and future studies could include additional replicates within and across sites. The scope of this study was limited to the fungi present in sampled carpet and one strain of *A. alternata*, and other fungal species could demonstrate different results. Carpet fibers analyzed via microscopy may not be a comprehensive representation of fungal morphology in the whole carpet sample for each condition tested. The fibers (1.25 mg) that were used in microscopy analyses were relatively small compared to the total number of fibers on the 5 cm × 5 cm carpet coupons. Furthermore, qPCR, confocal, and SEM imaging were all performed on one sample for each condition which means fungi may have been removed during confocal analysis and may have not been identified via qPCR or SEM. Additionally, the absorbent nature of the carpet fiber materials and their retention of the fluorescent stain may have obscured visualization of all fungal cells by fluorescence microscopy. This study did not take in account fungal growth on polyester carpet which has become a major fiber material in residential flooring [94]. In addition, carpet fiber pile heights were varied in these studies, and the effect of pile height on fungal growth is unknown. Each site sampled displayed different fungal growth patterns with ERH changes. This may be attributed to the chemical composition of each site's house dust, which was not analyzed in this study. In addition, qPCR values are reported in spore equivalents and will not account for differences in DNA extraction efficiency, amplification bias, or gene copy number between species [65,95]. Quantification of fungi by DNA is unable to determine fungal viability in the samples as well as fungal cell types (hyphae vs conidia). However, combining the DNA data with allergen levels can be useful since non-viable fungal fragments can still contain allergens. Standard limitations for fungal ITS sequencing such as amplification bias may cause error in the rank of fungal species in this analysis. We also analyzed dust from a limited number of homes for this study, and additional sites and geographic variability between sites could be further evaluated in future work. Direct qPCR spore quantity comparisons between total fungal DNA and *A. alternata* may not be accurately reflected due to differences in the gene region each primer set targeted. Total fungal primers amplified the ITS region while the *A. alternata* specific primers targeted the β-tubulin region.

5. Conclusions

We demonstrated that elevated ERH, fiber material, and dust presence all influence microbial growth in carpet. The most important factor that contributes to fungal growth in carpets as well as allergen levels is elevated moisture, followed by the presence of house dust. Olefin carpet fibers appear to be the best choice for minimizing fungal and *A. alternata* growth compared to nylon and wool fibers. Allergen sensitive individuals may consider solid floors over carpet since dust removal is easier and more effective, and resuspension rates for dust are lower. This would be especially important in areas of high moisture, such as a bathroom. These results have implications for future home design and maintenance of carpet that can potentially improve human health.

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Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

[1]. Nurmagambetov T, Kuwahara R, Garbe P, The economic burden of asthma in the United States, 2008-2013, Ann. Am. Thorac. Soc 15 (3) (2018) 348–356. [PubMed: 29323930]

[2]. Zahran HS, Bailey CM, Damon SA, Garbe PL, Breysse PN, Morbidity and mortality weekly report vital signs: asthma in children-United States, 2001-2016, MMWR (Morb. Mortal. Wkly. Rep.) 67 (5) (2018) 149–155. [PubMed: 29420459]

[3]. Leung DYM, Ledford DK, Bryant-Stephens T, Clinical reviews in allergy and immunology asthma disparities in urban environments, J. Allergy Clin. Immunol. 123 (6) (2009) 1199–1206. [PubMed: 19501229]

[4]. Klepeis NE, Nelson WC, Ott WR, Robinson JP, Tsang AM, Switzer P, Nehar JV, Hern SC, Engelmann WH, The National Human Activity Pattern Survey (NHAPS): a resource for assessing exposure to environmental pollutants, J. Expo. Sci. Environ. Epidemiol 11 (3) (2001) 231–252.

[5]. Lanphear BP, Kahn RS, Berger O, Auinger P, Bortnick SM, Nahhas RW, Contribution of residential exposures to asthma in US children and adolescents, J. Pediatr. 107 (6) (2001).

[6]. Mudarri DH, Valuing the economic costs of allergic rhinitis, acute bronchitis, and asthma from exposure to indoor dampness and mold in the US, J. Environ. Public Health 2016 (2016) 1–12.

[7]. Dannemiller KC, Gent JF, Leaderer BP, Peccia J, Indoor microbial communities: influence on asthma severity in atopic and nonatopic children, J. Allergy Clin. Immunol. 138 (1) (2016) 76–83, 10.1016/j.jaci.2015.11.027. [PubMed: 26851966]

[8]. Agarwal R, Gupta D, Severe asthma and fungi: current evidence, Med. Mycol. 49 (SI) (2011) S150–S157. [PubMed: 20662637]

[9]. Mendell M, Mendell MJ, Mirer AG, Cheung K, Tong M, Douwes J, Respiratory and allergic health effects of dampness, mold, and dampness-related agents: a review of the epidemiologic evidence, Environ. Heal. Perspect. Env. Heal. Perspect 119 (6) (2011) 748–756.

[10]. Pekkanen J, Hyvai inen A, Haverinen-Shaughnessy U, Korppi M, Putus T, Nevalainen, Moisture damage and childhood asthma: a population-based incident case-control study, Eur. Respir. J. 29 (3) (2007) 509–515. [PubMed: 17107993]

[11]. Institute of Medicine, Damp Buildings, Damp Indoor Spaces and Health, The National Academies Press, Washington D.C., 2004.

[12]. Simon-Nobbe B, Denk U, Poll V, Rid R, Breitenbach M, The spectrum of fungal allergy, Int. Arch. Allergy Immunol. 145 (1) (2008) 58–86. [PubMed: 17709917]

[13]. Vailes L, Sridhara S, Cromwell O, Weber B, Breitenbach M, Chapman M, Quantitation of the major fungal allergens, Alt a 1 and Asp f 1, in commercial allergenic products, J. Allergy Clin. Immunol. 107 (4) (2001) 641–646. [PubMed: 11295652]

[14]. Bowyer P, Fraczek M, Denning DW, Comparative genomics of fungal allergens and epitopes shows widespread distribution of closely related allergen and epitope orthologues, BMC Genom. 7 (1) (2006) 1–14.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

[15]. Hong SG, Cramer RA, Lawrence CB, Pryor BM, Alt a 1 allergen homologs from Alternaria and related taxa: analysis of phylogenetic content and secondary structure, Fungal Genet. Biol. 42 (2) (2005) 119–129. [PubMed: 15670710]

[16]. Saenz-de-Santamaria M, Postigo I, Gutierrez-Rodriguez A, Cardona G, Guisantes JA, Asturias J, Martinez J, The major allergen of Alternaria alternata (Alt a 1) is expressed in other members of the Pleosporaceae family, Mycoses 49 (2) (2006) 91–95. [PubMed: 16466440]

[17]. Teifoori F, Shams-Ghahfarokhi M, Razzaghi-Abyaneh M, Martinez J, Gene profiling and expression of major allergen Alt a 1 in Alternaria alternata and related members of the Pleosporaceae family, Rev. Iberoam. De. Micol 36 (2) (2019) 66–71.

[18]. Gutierrez-Roriduez A, Postigo I, Guisantes JA, Sunen E, Martinez J, Identification of allergens homologous to Alt a 1 from Stemphylium botryosum and Ulocladium botrytis, Med. Mycol. 49 (2011) 892–896. [PubMed: 21504377]

[19]. Salo PM, Yin M, Arbes SJ, Cohn RD, Sever M, Muilenberg M, Burge HA, London SJ, Zeldin DC, Dustborne Alternaria alternata antigens in U.S. Homes: results from the national survey of lead and allergens in housing, J. Allergy Clin. Immunol. 116 (3) (2015) 623–629.

[20]. Schmechel D, Green BJ, Blachere FM, Janotka E, Beezhold DH, Environmental allergens Analytical bias of cross-reactive polyclonal antibodies for environmental immunoassays of Alternaria alternata, J. Allergy Clin. Immunol. 121 (3) (2008) 763–771, 10.1016/j.jaci.2007.09.046. [PubMed: 18036643]

[21]. Perzanowski MS, Sporik R, Squillace SP, Gelber LE, Call R, Carter M, Platts-Mills TAE, Association of sensitization to Alternaria allergens with asthma among school-age children, J. Allergy Clin. Immunol. 101 (5) (1998) 626–632, 10.1016/S0091-6749(98)70170-8. [PubMed: 9600499]

[22]. Licorish K, Novey HS, Kozak P, Fairshter RD, Wilson AF, Role of Alternaria and Penicillium spores in the pathogenesis of asthma, J. Allergy Clin. Immunol. 76 (6) (1985) 819–825. [PubMed: 4067131]

[23]. Andersson M, Downs S, Mitakakis T, Leuppi J, Marks G, Natural exposure to Alternaria spores induces allergic rhinitis symptoms in sensitized children, Pediatr. Allergy Immunol. 14 (2) (2003), 100–105. [PubMed: 12675755]

[24]. Bush RK, Prochnau JJ, Alternaria-induced asthma, J. Allergy Clin. Immunol. 113 (2) (2004) 227–234. [PubMed: 14767434]

[25]. Salo PM, Arbes SJ, Sever M, Jaramillo R, Cohn RD, London SJ, Zeldin DC, Exposure to Alternaria alternata in US homes is associated with asthma symptoms, J. Allergy Clin. Immunol. 118 (4) (2006) 892–898, 10.1016/j.jaci.2006.07.037. [PubMed: 17030243]

[26]. Pasanen P, Korpi A, Kalliokoski P, Pasanen AL, Growth and volatile metabolite production of Aspergillus versicolor in house dust, Environ. Int 23 (4) (1997) 425–432.

[27]. Haleem Khan AA, Mohan Karuppayil S, Fungal pollution of indoor environments and its management, Saudi J. Biol. Sci. 19 (4) (2012) 405–426. [PubMed: 23961203]

[28]. Miller JD, Fungi as contaminants in indoor air, Atmos. Environ. 26 (12) (1992) 2163–2172, 10.1016/0960-1686(92)90404-9.

[29]. Fischer G, Dott W, Relevance of airborne fungi and their secondary metabolites for environmental, occupational and indoor hygiene, Arch. Microbiol. 179 (2) (2003) 75–82. [PubMed: 12560984]

[30]. Mendell MJ, Macher JM, Kumagai K, Measured moisture in buildings and adverse health effects: a review, Indoor Air 28 (4) (Jul. 2018) 488–499. [PubMed: 29683210]

[31]. Dannemiller KC, Weschler CJ, Peccia J, Fungal and bacterial growth in floor dust at elevated relative humidity levels, Indoor Air 27 (2) (2017) 354–363, 10.1111/ina.12313. [PubMed: 27272645]

[32]. Hagerty B, Dannemiller KC, Peccia J, Gene expression of indoor fungal communities under damp building conditions: implications for human health, Indoor Air (2018) 1–11, 10.1111/ina.12459, 00.

[33]. Green BJ, Mitakakis TZ, Tovey ER, Allergen detection from 11 fungal species before and after germination, J. Allergy Clin. Immunol. Ill (2) (2003) 285–289, 10.1067/mai.2003.57.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

[34]. Mitakakis TZ, Barnes C, Tovey ER, Wales NS, City K, Spore germination increases allergen release from Alternaria, J. Allergy Clin. Immunol. 107 (2) (2001) 388–390, 10.1067/mai.2001.112602. [PubMed: 11174210]

[35]. Vlasenko A, Sjogren S, Weingartner E, Gaggeler HW, Ammann M, Generation of submicron Arizona test dust aerosol: chemical and hygroscopic properties, Aerosol Sci. Technol. 39 (5) (2005) 452–460.

[36]. Joshi N, Romanias MN, Riffault V, Thevenet F, Investigating water adsorption onto natural mineral dust particles: linking DRIFTS experiments and BET theory, Aeolian Research 27 (2017) 35–45.

[37]. Boor BE, Siegel JA, Novoselac A, Monolayer and multilayer particle deposits on hard surfaces: literature review and implications for particle resuspension in the indoor environment, Aerosol Sci. Technol. 47 (8) (2013) 831–847.

[38]. Andersen B, Frisvad JC, Søndergaard I, Rasmussen IS, Larsen LS, Associations between fungal species and water-damaged building materials, Appl. Environ. Microbiol. 77 (12) (2011) 4180–4188. [PubMed: 21531835]

[39]. Nielsen KF, Holm G, Uttrup LP, Nielsen PA, Mould growth on building materials under low water activities. Influence of humidity and temperature on fungal growth and secondary metabolism, Int. Biodeterior. Biodegrad. 54 (4) (2004) 325–336.

[40]. US EPA, Climate change indicators: coastal flooding [Online] Available: https://www.epa.gov/climate-indicators/climate-change-indicators-coastal-flooding [Accessed: 18-June-2019].

[41]. Adams RI, Miletto M, Taylor JW, Bruns TD, Dispersal in microbes: fungi in indoor air are dominated by outdoor air and show dispersal limitation at short distances, ISME J. 7 (2013) 1262–1273. [PubMed: 23426013]

[42]. Górny RL, Reponen T, Willeke K, Schmechel D, Robine E, Boissier M, Grinshpun SA, Fungal fragments as indoor air biocontaminants, Appl. Environ. Microbiol. 68 (7) (2002) 3522–3531, 10.1128/AEM.68.7.3522-3531.2002. [PubMed: 12089037]

[43]. Kavanagh K, Wiley J, Fungi Biology and Applications, 3rd, Wiley-Blackwell, Hoboken, NJ, 2017.

[44]. Lax S, Cardona C, Zhao D, Winton VJ, Goodney G, Gao P, Gottel N, Hartmann EM, Henry C, Thomas PM, Kelley ST, Stephens B, Gilbert JA, Microbial and metabolic succession on common building materials under high humidity conditions, Nat. Commun 10 (1) (2019), 10.1038/s41467-019-09764-z.

[45]. Dannemiller KC, Gent JF, Leaderer BP, Peccia J, Influence of housing characteristics on bacterial and fungal communities in homes of asthmatic children, Indoor Air 26 (2) (2016) 179–192, 10.1111/ina.12205. [PubMed: 25833176]

[46]. Chase J, Fouquier J, Zare M, Sonderegger DL, Knight R, Kelley ST, Siegel J, Caporaso JG, Geography and Location Are the Primary Drivers of Office Microbiome Composition, vol. 1, ASM, 2016, 10.1128/mSystems.00022-16 no. 2, sp. e00022-16.

[47]. Qian J, Hospodsky D, Yamamoto N, Nazaroff WW, Peccia J, Size-resolved emission rates of airborne bacteria and fungi in an occupied classroom, Indoor Air 22 (4) (2012) 339–351. [PubMed: 22257156]

[48]. "Research, Resources - CRI [Online]. Available: https://carpet-rug.org/resources/research-and-resources/. Accessed: 15-Nov-2018.

[49]. Cunningham MJ, Modelling of some dwelling internal microclimates, Build. Environ. 34 (5) (1999) 523–536, 10.1016/S0360-1323(98)00044-4.

[50]. Lave L, Conway-Schempf N, Harvey J, Hart D, Bee T, Maccracken C, Applications and Implementations: Recycling Postconsumer Nylon Carpet A Case Study of the Economics and Engineering Issues Associated with Recycling Postconsumer Goods KeY-Jvords Carpet Recycling Landfill Cover Materials Properties, 1998.

[51]. Realff M, Systems planning for carpet recycling, in: Recycling in Textiles, Woodland Publishing, 2006, pp. 46–57, 10.1533/9781845691424.1.46.

[52]. Green JL, Can bioinformed design promote healthy indoor ecosystems? Indoor Air 24 (2) (2014) 113–115. [PubMed: 24628783]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

[53]. Dannemiller KC, Moving towards a robust definition for a 'healthy' indoor microbiome, mSystems 4 (3) (2019), 10.1128/mSystems.00074-19.

[54]. Wolf DC, Dao TH, Scott HD, Lavy TL, Influence of sterilization methods on selected soil microbiological, physical, and chemical properties, J. Environ. Qual. 18 (1) (2010) 39.

[55]. Koch HH, Pimsler M, Histology evaluation of Uvitex 2B: a nonspecific fluorescent stain for detecting and identifying fungi and algae in tissue, Lab. Med. 18 (9) (1987) 603–606, 10.1093/labmed/18.9.603.

[56]. Hospodsky D, Yamamoto N, Peccia J, Accuracy, precision, and method detection limits of quantitative PCR for airborne bacteria and fungi, Appl. Environ. Microbiol. 76 (21) (2010) 7004–7012, 10.1128/AEM.01240-10. [PubMed: 20817798]

[57]. Zhou G, Whong WZ, Ong T, Chen B, Development of a fungus-specific PCR assay for detecting low-level fungi in an indoor environment, Mol. Cell. Probes 14 (6) (2000) 339–348. [PubMed: 11090263]

[58]. Kordalewska M, Brillowska-Dqbrowska A, Jagielski T, Dworecka-Kaszak B, PCR and real-time PCR assays to detect fungi of Alternaria alternata species, ABP. 62 (4) (2015) 707–712, 10.18388/abp.2015_1112.

[59]. Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA, Chen W, Fungal Barcoding Consortium, Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi, Proc. Natl. Acad. Sci. U.S.A. 109 (16) (2012) 1–6, 10.1073/pnas.1117018109.

[60]. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Pẽna AG, Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE, Louzupone CA, McDonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Turnbaugh PJ, Walters WA, Widmann J, Yatsunenko T, Zaneveld J, Knight R, QIIME allows analysis of high-throughput community sequencing data, Nat. Methods 7 (5) (2010) 335–336, 10.1038/nmeth.f.303. [PubMed: 20383131]

[61]. St John J, SeqPrep [Online] Available: https://github.com/jstjohn/SeqPrep. Accessed: 24-Sept-2019.

[62]. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ, Basic local alignment search tool, J. Mol. Biol. 215 (3) (1990) 403–410. [PubMed: 2231712]

[63]. Abarenkov K, Nilsson RH, Larsson KH, Alexander IJ, Eberhardt U, Erland S, Høiland K, Kjøller R, Larsson E, Pennanen T, Sen R, Taylor AFS, Tedersoo L, Ursing BM, Vrålstad T, Liimatainen K, Peintner U, KõLjalg U, The UNITE database for molecular identification of fungi - recent updates and future perspectives, New Phytol. 186 (2) (2010) 281–285, 10.1111/j.1469-8137.2009.03160.x. [PubMed: 20409185]

[64]. Dannemiller KC, Reeves D, Bibby K, Yamamoto N, Peccia J, Fungal high-throughput taxonomic identification tool for use with next-generation sequencing (FHiTINGS), J. Basic Microbiol. 54 (4) (2014) 315–321, 10.1002/jobm.201200507. [PubMed: 23765392]

[65]. Dannemiller KC, Lang-Yona N, Yamamoto N, Rudich Y, Peccia J, Combining real-time PCR and next-generation DNA sequencing to provide quantitative comparisons of fungal aerosol populations, Atmos. Environ. 84 (2014) 113–121, 10.1016/j.atmosenv.2013.11.036.

[66]. Renstro A, Karlsson A-S, Tovey E, Nasal air sampling used for the assessment of occupational allergen exposure and the efficacy of respiratory protection, Clin. Exp. Allergy 32 (12) (2002) 1769–1775, 10.1046/j.1365-2222.2002.01545.x. [PubMed: 12653170]

[67]. Samet JM, Spengler JD, Indoor environments and health: moving into the 21st century, Am. J. Publ. Health 93 (9) (2003) 1489–1493.

[68]. Haleem Khan AA, Mohan Karuppayil S, Fungal pollution of indoor environments and its management, Saudi J. Biol. Sci. 19 (4) (2012) 405–426. [PubMed: 23961203]

[69]. Korpi A, Pasanen A-L, Pasanen P, Kalliokoski P, Microbial growth and metabolism in house dust, Int. Biodeterior. Biodegrad. 40 (1) (1997) 19–27.

[70]. Bope A, Haines SR, Hegarty B, Weschler CJ, Peccia J, Dannemiller KC, Degradation of phthalate esters in floor dust at elevated relative humidity, Environ. Sci.: Processes Impacts 21 (2019) 1268–1279, 10.1039/C9EM00050J.

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[71]. Araki A, Kanazawa A, Kawai T, Eitaki Y, Morimoto K, Nakayama K, Shibata E, Tanaka M, Takigawa T, Yoshimura T, Chikara H, Saijo Y, Kishi R, The relationship between exposure to microbial volatile organic compound and allergy prevalence in single-family homes, Sci. Total Environ. 423 (2012) 18–26. [PubMed: 22405561]

[72]. Harving H, Korsgaard J, Dahl R, Clinical efficacy of reduction in house-dust mite exposure in specially designed, mechanically ventilated “healthy” homes, Eur Ann Allergy Clin Immunol 49 (10) (1994) 866–870.

[73]. Franke DL, Cole EC, Leese KE, Foarde KK, Berry MA, Cleaning for improved indoor air quality: an initial assessment of effectiveness, Indoor Air 7 (1) (1997) 41–54.

[74]. Causer S, Shorter C, Sercombe J, Effect of floorcovering construction on content and vertical distribution of house dust mite allergen, der p I, Appl. Occup. Environ. Hyg 3 (4) (2007) 161–168.

[75]. Qian J, Ferro AR, Resuspension of dust particles in a chamber and associated environmental factors, Aerosol Sci. Technol. 42 (7) (2008) 566–578.

[76]. Tian Y, Sul K, Qian J, Mondal S, Ferro AR, A comparative study of walking-induced dust resuspension using a consistent test mechanism, Indoor Air 24 (6) (2014) 592–603. [PubMed: 24605758]

[77]. Salares VR, Hinde CA, Miller JD, Analysis of settled dust in homes and fungal glucan in air particulate collected during HEPA vacuuming, Indoor Built Environ. 18 (6) (2009) 485–491.

[78]. Seo Y, Han T, Assessment of penetration through vacuum cleaners and recommendation of wet cyclone technology, J. Air Waste Manag. Assoc. 63 (4) (2013) 453–461. [PubMed: 23687730]

[79]. Chung E, Yiacoumi S, Lee I, Tsouris C, The role of the electrostatic force in spore adhesion, Environ. Sci. Technol. 44 (16) (2010) 6209–6214. [PubMed: 20666490]

[80]. Epa U, A Brief Guide to Mold, Moisture, and Your Home EPA-402-K-02-003, 9 2010, 2012.

[81]. Olaf RAS, Adan CG, Fundamentals of Mold Growth in Indoor Environments and Strategies for Healthy Living, Wageningen Academic Publishers, The Netherlands, 2011.

[82]. Călin M, Constantinescu-Aruxandei D, Alexandrescu E, Râut I, Doni MB, Arsene M, Oancea F, Jecu L, Lazar V, Degradation of keratin substrates by keratinolytic fungi, Electron. J. Biotechnol. 28 (2017) 101–112.

[83]. Bush RK, Prochnau JJ, Alternaria-induced asthma, J. Allergy Clin. Immunol 113 (2) (2004) 227–234. [PubMed: 14767434]

[84]. Simon-Nobbe B, Denk U, Pöll V, Rid R, Breitenbach M, The spectrum of fungal allergy, Int. Arch. Allergy Immunol. 145 (1) (2008) 58–86. [PubMed: 17709917]

[85]. Green BJ, Mitakakis TZ, Tovey ER, Allergen detection from 11 fungal species before and after germination, J. Allergy Clin. Immunol. Ill (2) (2003) 285–289.

[86]. Brito FF, Alonso AM, Carnes J, Martin-Martin R, Fernandez-Caldas E, Galindo PA, Alfaya T, Amo-Salas M, Correlation between Alt a 1 levels and clinical symptoms in alternaria alternata-monosensitized patients, J Investig. Allergol. Clin. Immunol. 22 (3) (2012) 154–159.

[87]. Li DW, Kendrick B, Indoor aeromycota in relation to residential characteristics and allergic symptoms, Mycopathologia 131 (3) (1995) 149–157. [PubMed: 8587581]

[88]. Andersen B, Frisvad JC, Søndergaard I, Rasmussen IS, Larsen LS, Associations between fungal species and water-damaged building materials, Appl. Environ. Microbiol. 77 (12) (2011) 4180–4188. [PubMed: 21531835]

[89]. Hertzen T, Leena von Hanski, Ilkka and Haahtela, “Natural Immunity Biodiversity loss and inflammatory diseases are two global megatrends that might be related, Eur. Mol. Biol. Organ. EMBO reports 12 (11) (2011) 1089–1093.

[90]. von Hertzen L, Beutler B, Bienenstock J, Blaser M, Cani PD, Eriksoon J, Färkkilä M, Haahtela T, Hanski I, Jenmalm MC, Kere J, Knip M, Kontula K, Koskenvuo M, Ling C, Mandrup-Poulsen T, von Mutius E, Mäkelä MJ, Paunio T, Pershagen G, Renz H, Rook G, Saarela M, Vaarala O, Veldhoen M, de Vos WM, Helsinki alert of biodiversity and heath, Thomas Mandrup-Poulsen 47 (3) (2015) 218–225.

[91]. Kembel SW, Meadow JF, O’Connor TK, Mhuireach G, Northcutt D, Kline J, Moriyama M, Brown GZ, Bohannan BJM, Green JL, Architectural Design Drives the Biogeography of Indoor Bacterial Communities, PLoS One 9 (1) (2014), e87093. [PubMed: 24489843]

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[92]. Adams RI, Miletto M, Taylor JW, Bruns TD, The diversity and distribution of fungi on residential surfaces, PLoS One 8 (11) (2013) 78866.

[93]. Kembel SW, et al., Architectural design influences the diversity and structure of the built environment microbiome, ISME J. 6 (8) (2012) 1469–1479. [PubMed: 22278670]

[94]. Helm D, Fiber: independent producer update [Online] Available: https://www.floordaily.net/floorfocus/fiber-independent-producer-update--mar-2016 [Accessed: 24-Sept-2019].

[95]. Herrera ML, Vallor AC, Gelfond JA, Patterson TF, Wickes BL, Strain-dependent variation in 18S ribosomal DNA copy numbers in aspergillus fumigatus,, J. Clin. Microbiol. 47 (5) (2009) 1325–1332. [PubMed: 19261786]

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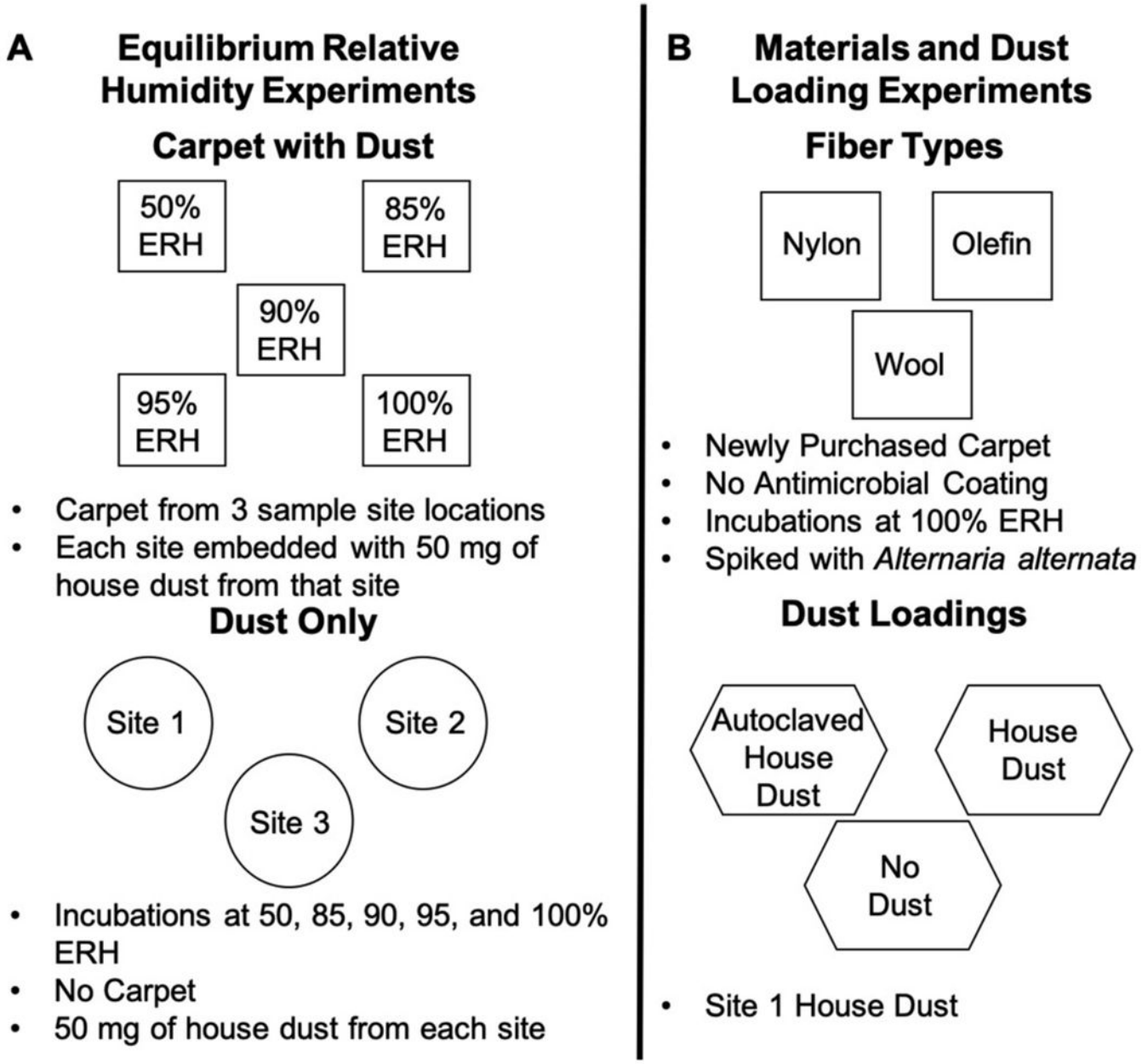

Fig. 1.

Summary of samples and conditions: (A) Study design for the effects of relative humidity in carpet embedded with dust and the dust by itself, (B) study design for the effect of common fiber materials wool, nylon, and olefin, and study design for the effect of autoclaved house dust, unaltered house dust, and no dust (B). Each square represents a 5 cm × 5 cm carpet coupon. Dust was distributed in center of carpet coupon before embedding. Each circle represents 50 mg of house dust only. All analyses were done with one physical carpet coupon and with triplicate qPCR measurements.

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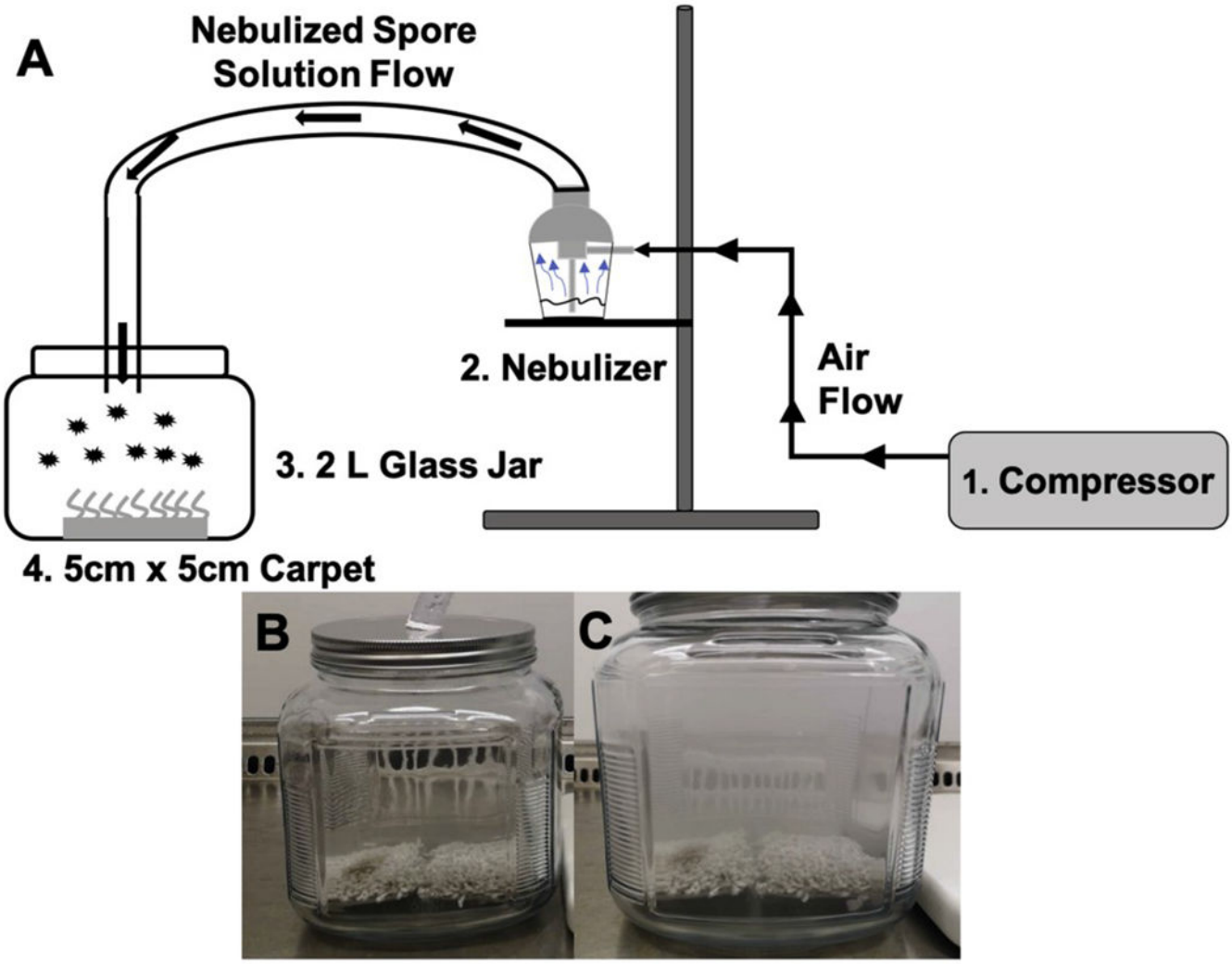

Fig. 2.

(A) Schematic of Inoculation setup. A compressor forces air into the nebulizer containing the *A. alternata* spore suspension. The nebulized solution exits the nebulizer and flows into the glass jar onto the carpet in the bottom. (B) Glass jar at time = 0 and (C) Glass jar at time = 10 min.

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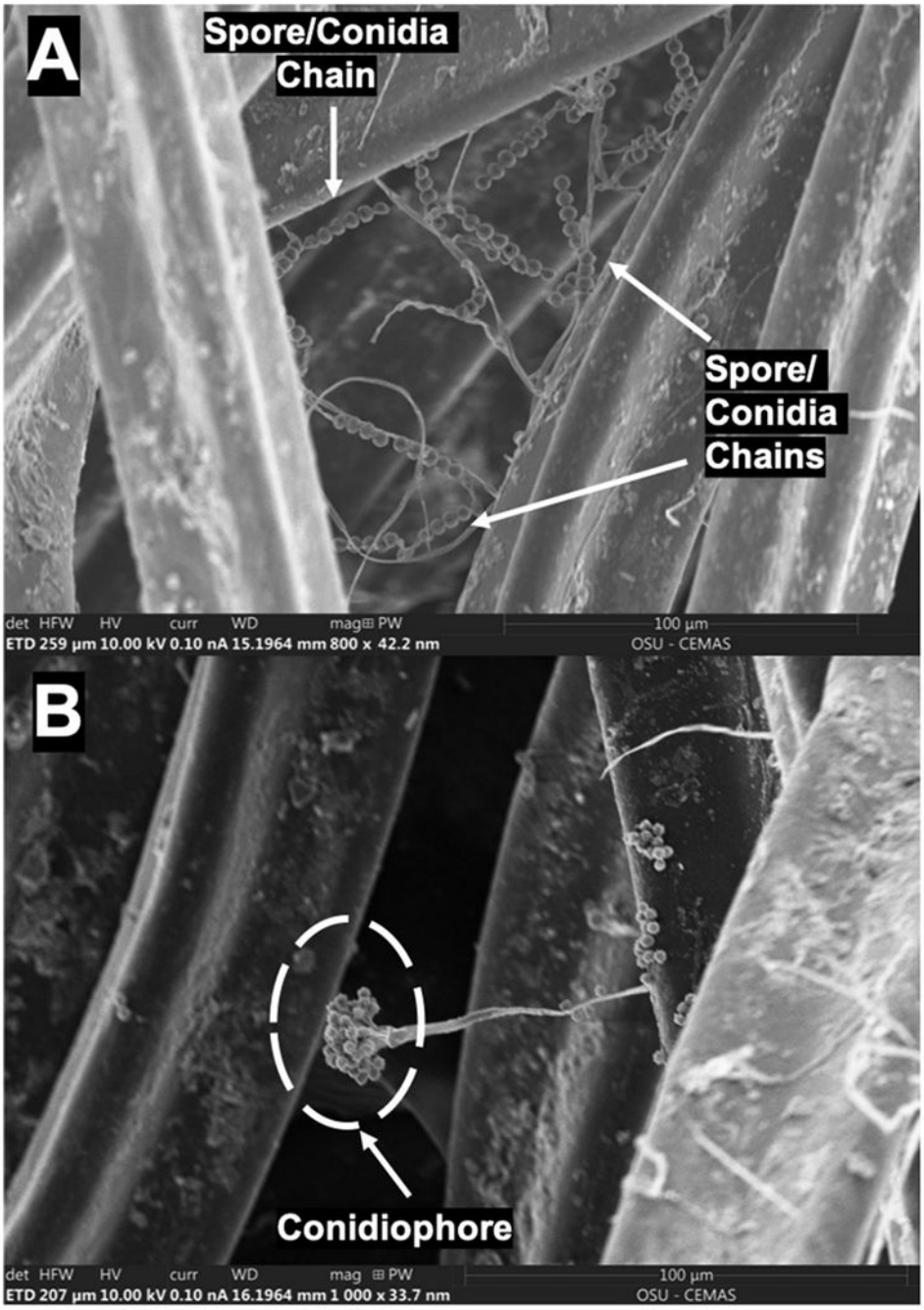

Fig. 3.

SEM images of samples incubated at 95% ERH and 25 °C for 2 weeks showing (A) spore/conidia chains and (B) conidiophores which are signs of fungal asexual reproduction. Both images were from Site 2 ERH experiment (Fig. 1A).

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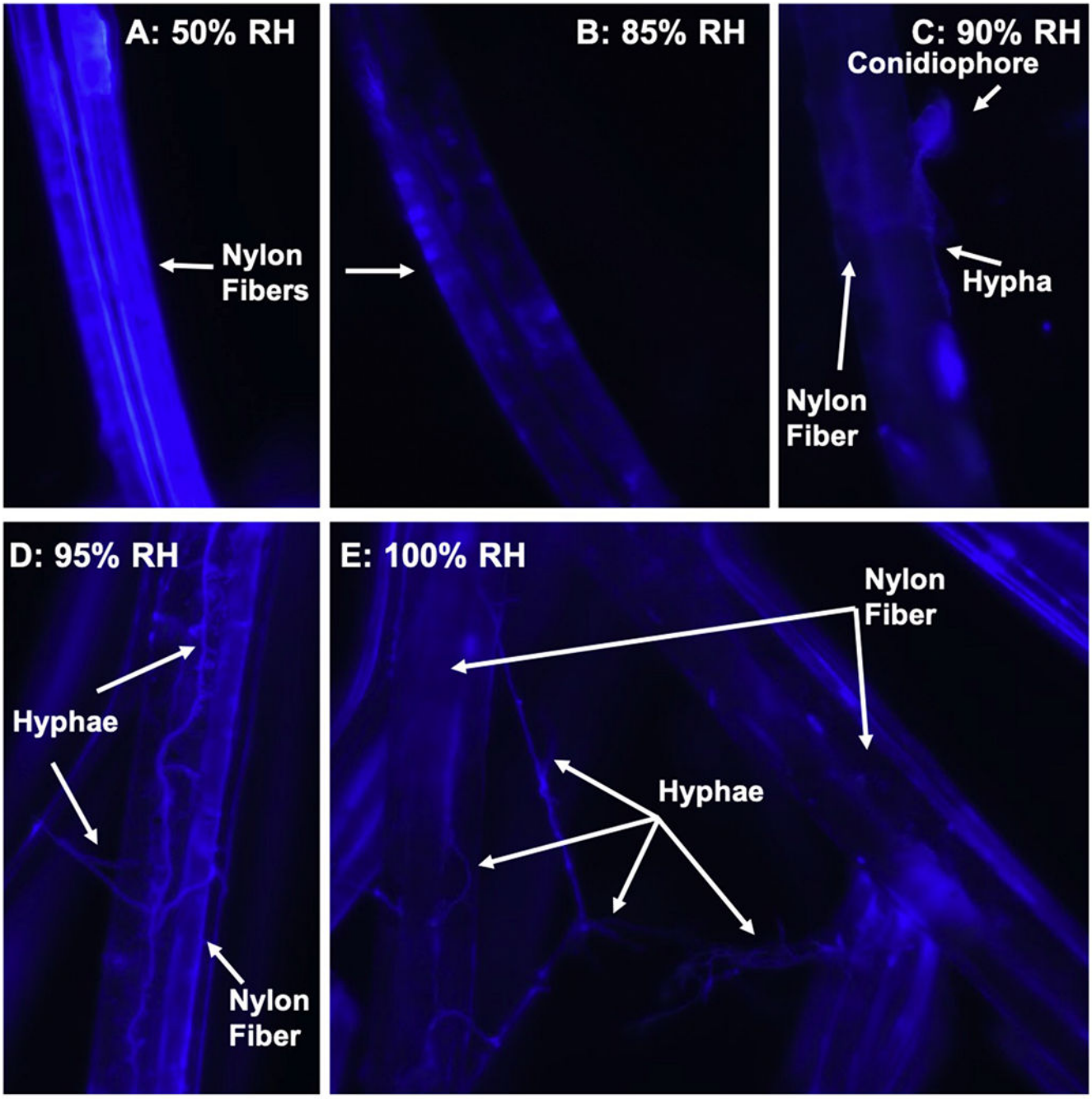

Fig. 4.

Confocal images of vegetative and reproductive fungal structures on nylon carpet fibers (Site 3). Samples were incubated at 25 °C for 2 weeks at (A) 50%, (B) 85%, (C) 90%, (D) 95%, and (E) 100% ERH conditions. Samples were fixed with 4% PFA, stained with Uvitex 2B, and gently washed with PBS.

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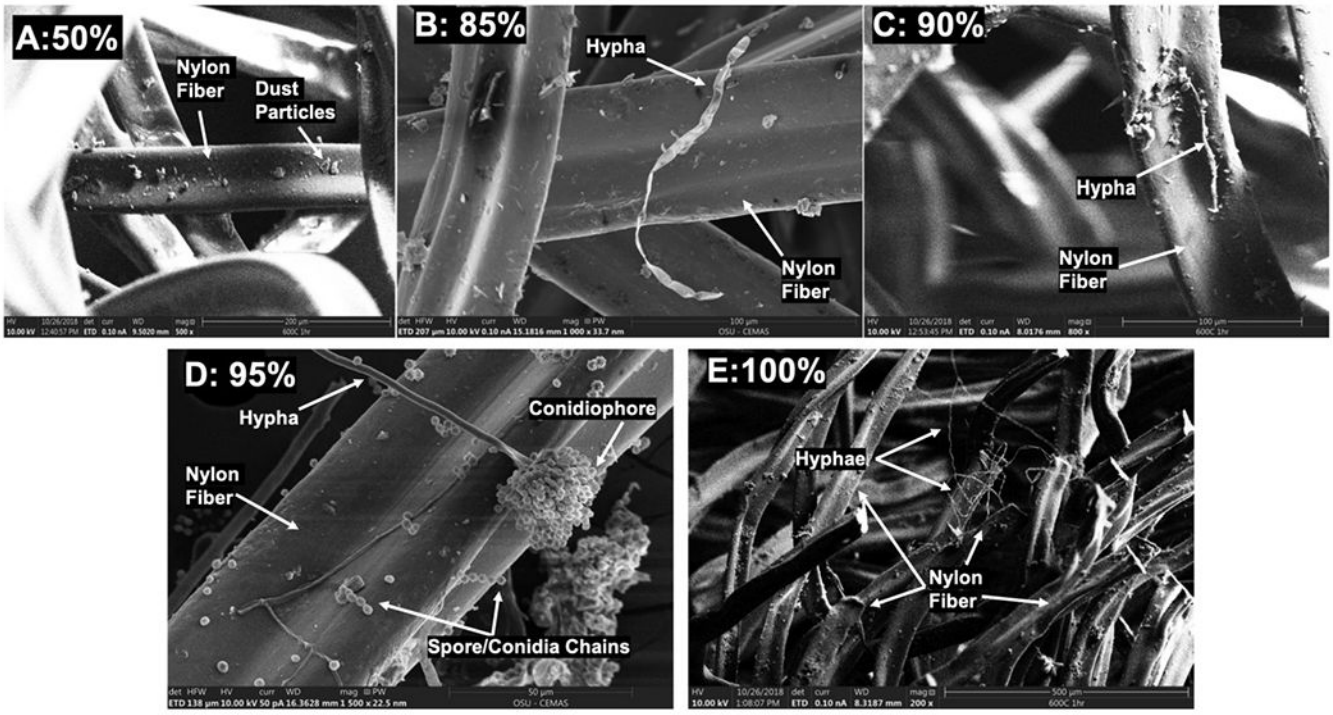

Fig. 5.

SEM images of (A) dust on fibers, no growth at 50% ERH, (B) small hyphae on fiber at 85% ERH (C) spores on fiber with small hyphal structures at 90% ERH, (D) spores, large hyphae, and conidiophores at 95% ERH, and (E) large hyphal networks and spores on fibers at 100% ERH. Samples were incubated at 25 °C for 2 weeks. Images A – C are from Site 1, Image D is from Site 2, and Image E is from Site 3.

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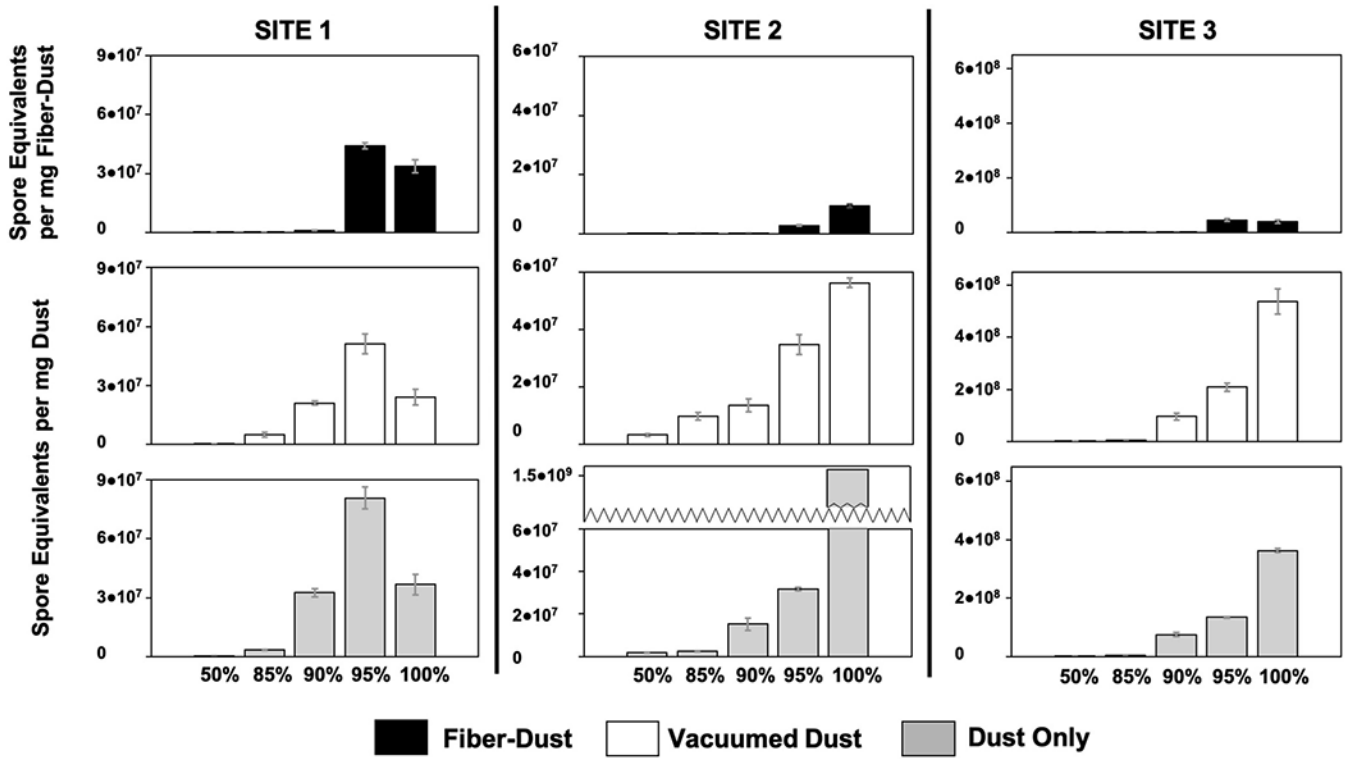

Fig. 6.

Total fungal DNA qPCR results for Sites 1–3. Samples from each site were incubated at 25 °C for two weeks at each ERH condition and embedded with house dust from their respective sites. “Fiber-Dust” samples were a combination of carpet fibers and dust extracted together, vacuumed dust was incubated in carpet and then vacuumed, and the dust only samples were never embedded in a carpet. All samples are done in triplicate and at 100X dilution. Error bars displayed are standard deviation of triplicate samples. $P < 0.05$ for all quantities within each site.

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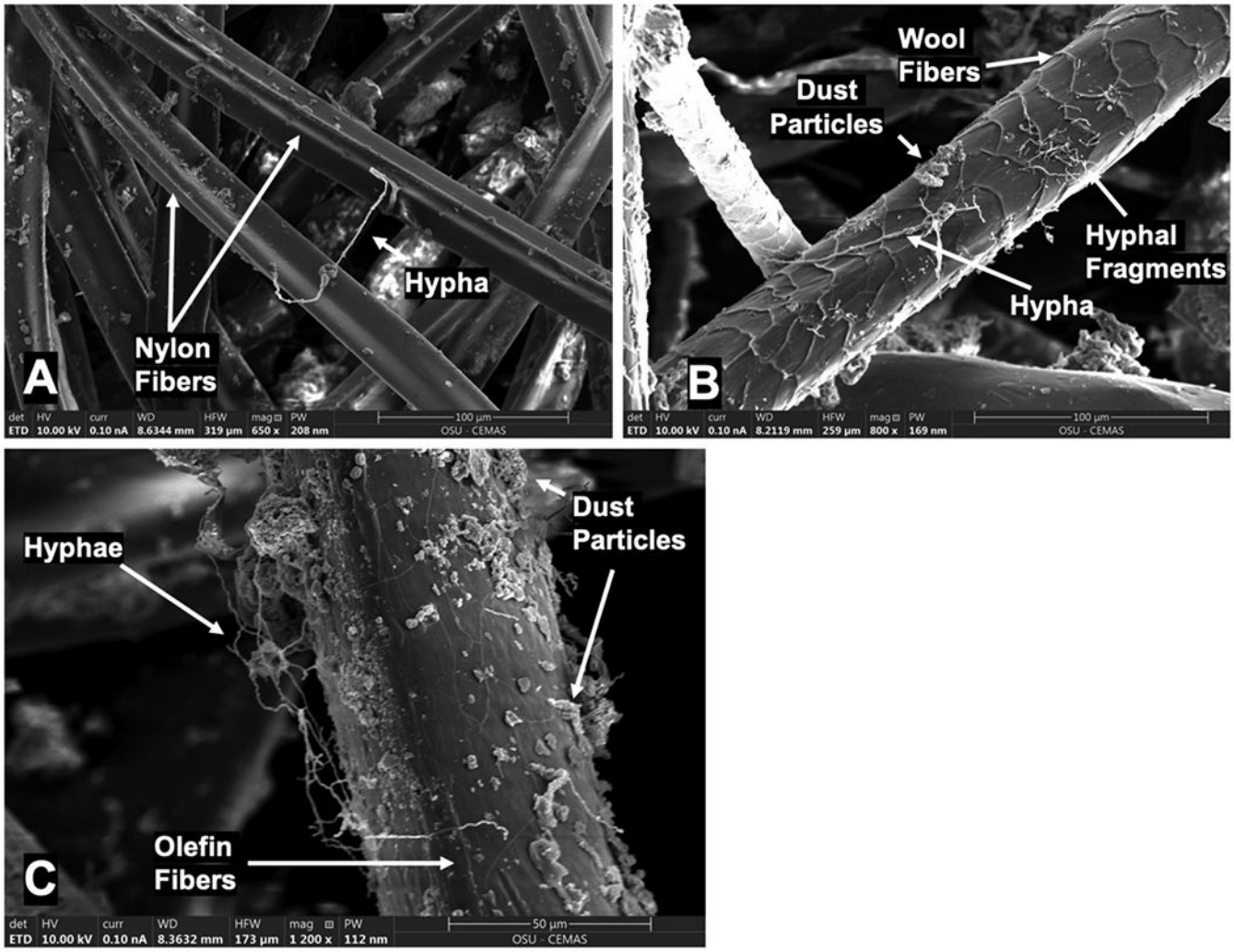

Fig. 7.

SEM images of *A. alternata* on (A) nylon, (B) wool, and (C) olefin carpet fibers. All samples were incubated at 25 °C for 2 weeks at 100% ERH and embedded with Site 1 autoclaved house dust.

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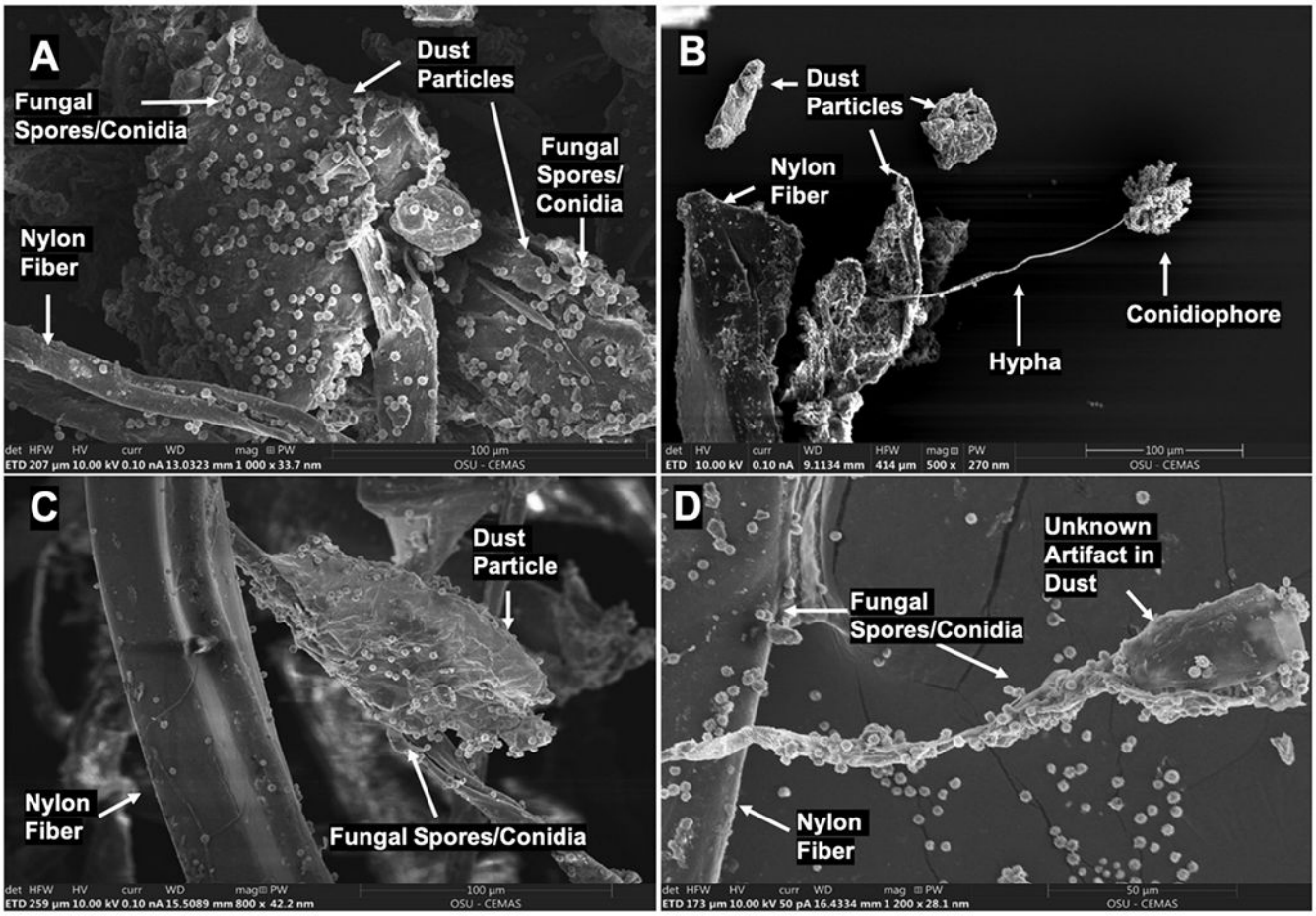

Fig. 8.

House Dust and fungal growth at 100% ERH. (A) fungal spores on dust particle, (B) hyphae with reproductive structures resembling *Aspergillus* conidiophore associated house dust at 100% ERH, (C) fungal spores on dust particle at 100% ERH, and (D) fungal spore attached to possible hair fiber or dust strand at 95% ERH.

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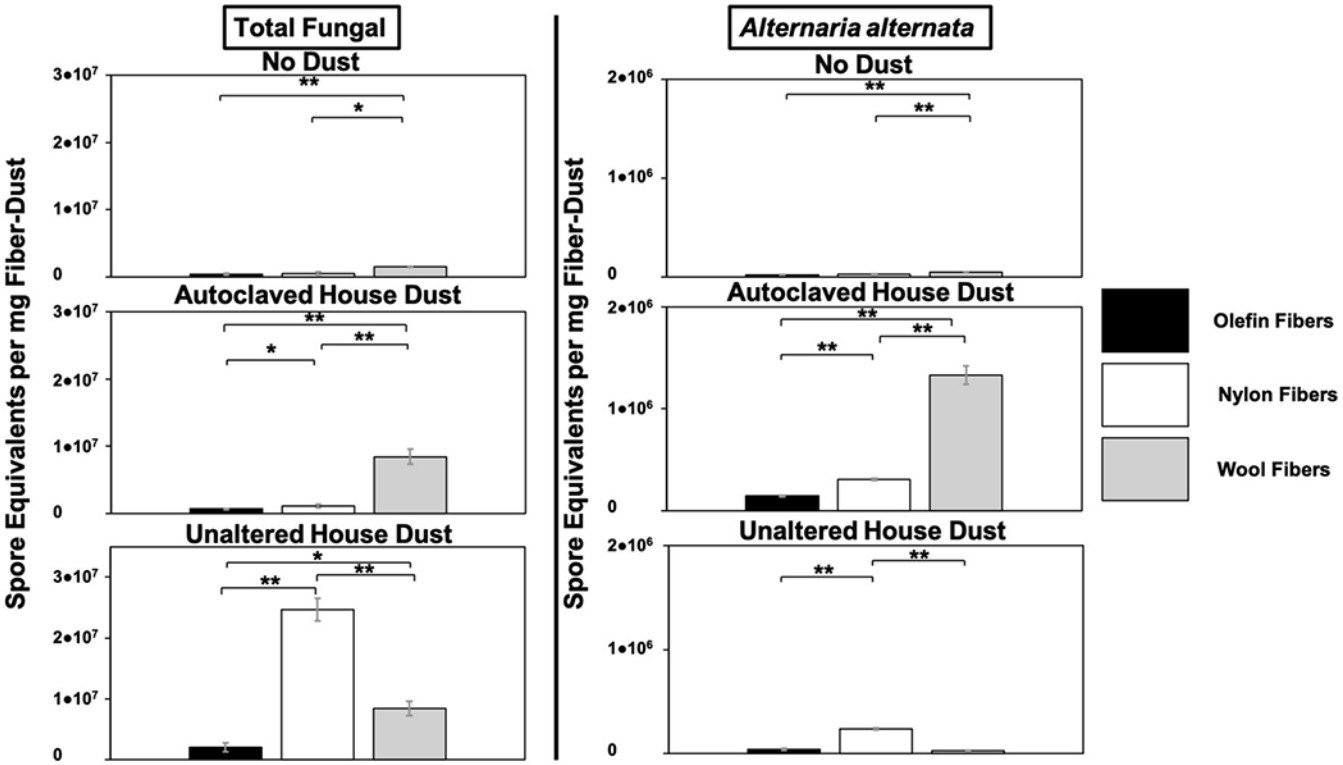

Fig. 9.

qPCR results for dust loading and carpet fiber materials. Total fungal and *A. alternata* specific quantities were measured for all samples. Error bars displayed are standard deviation of three technical replicate qPCR measurements for each single carpet coupon. All samples incubated at 25 °C for 2 weeks at 100% ERH. Statistical data can be found in Table S5. *P < 0.05, **P < 0.005.

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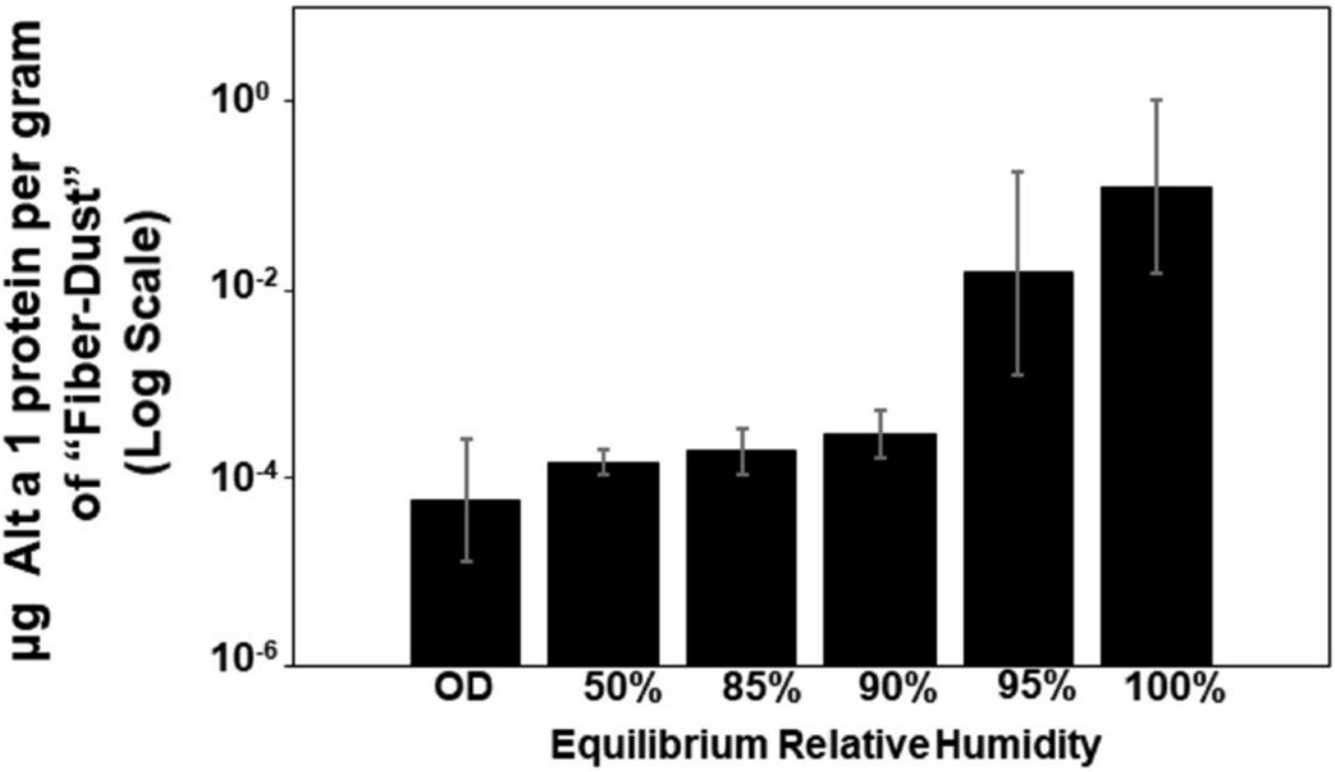

Fig. 10.

Alt a 1 allergen quantity for all site's original dust (OD) and after incubations at 50, 85, 90, 95, and 100% ERH. Quantities represent the average of all three sites and error bars shown represent the 95% confidence interval. All samples were incubated for 2 weeks at 25 °C. Alt a 1 allergen ELISA measurements were made on "Fiber-Dust" samples for each site and ERH condition, while OD measurements were dust only samples with no incubation. Statistical analyses of Alt a 1 measurement are outlined in Table S6.

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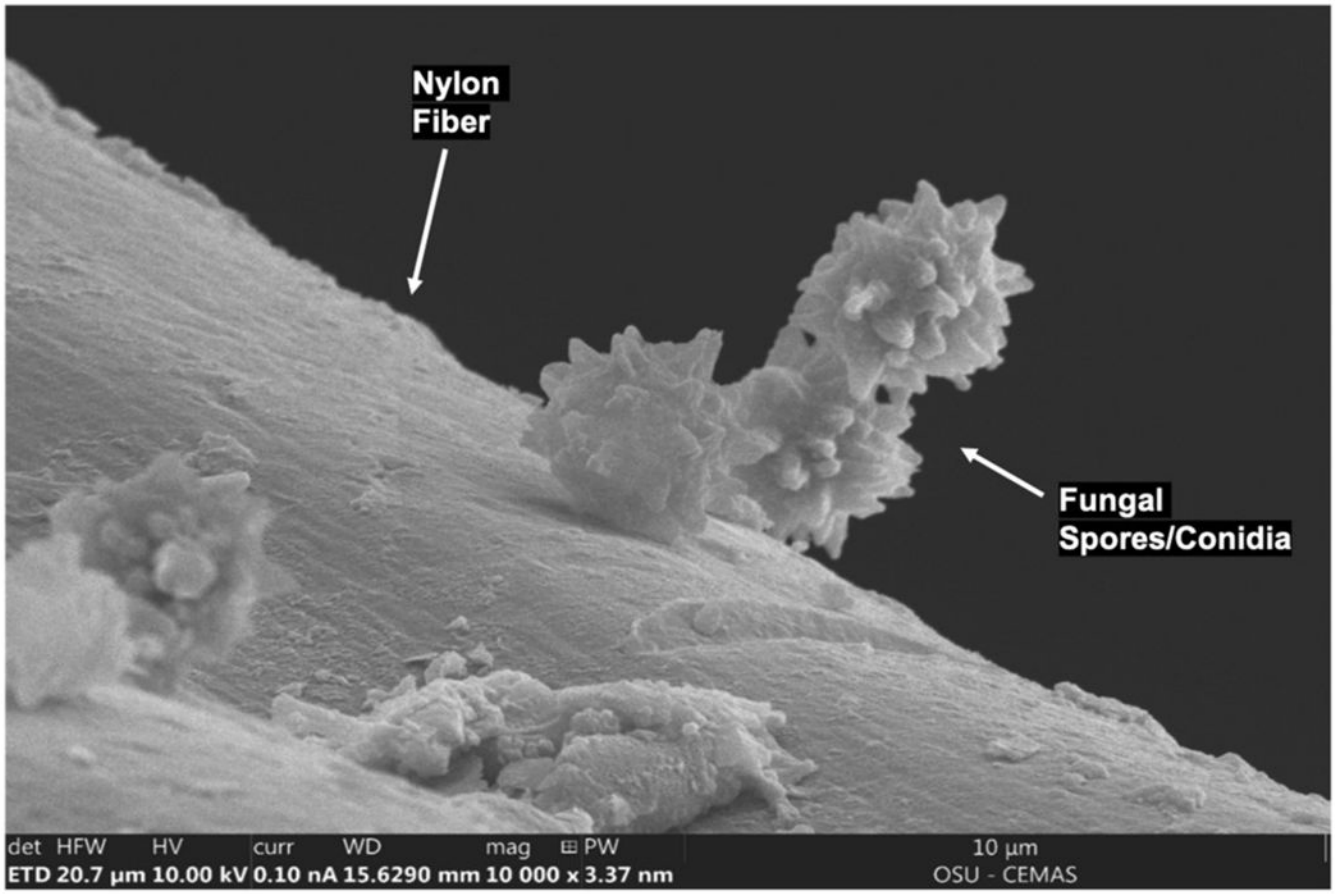

Fig. 11.

Putative *Aspergillus sydowii* spores resting on nylon carpet fiber. Carpet embedded with house dust from Site 2. Incubated for 2 weeks at 25 °C and 95% RH.

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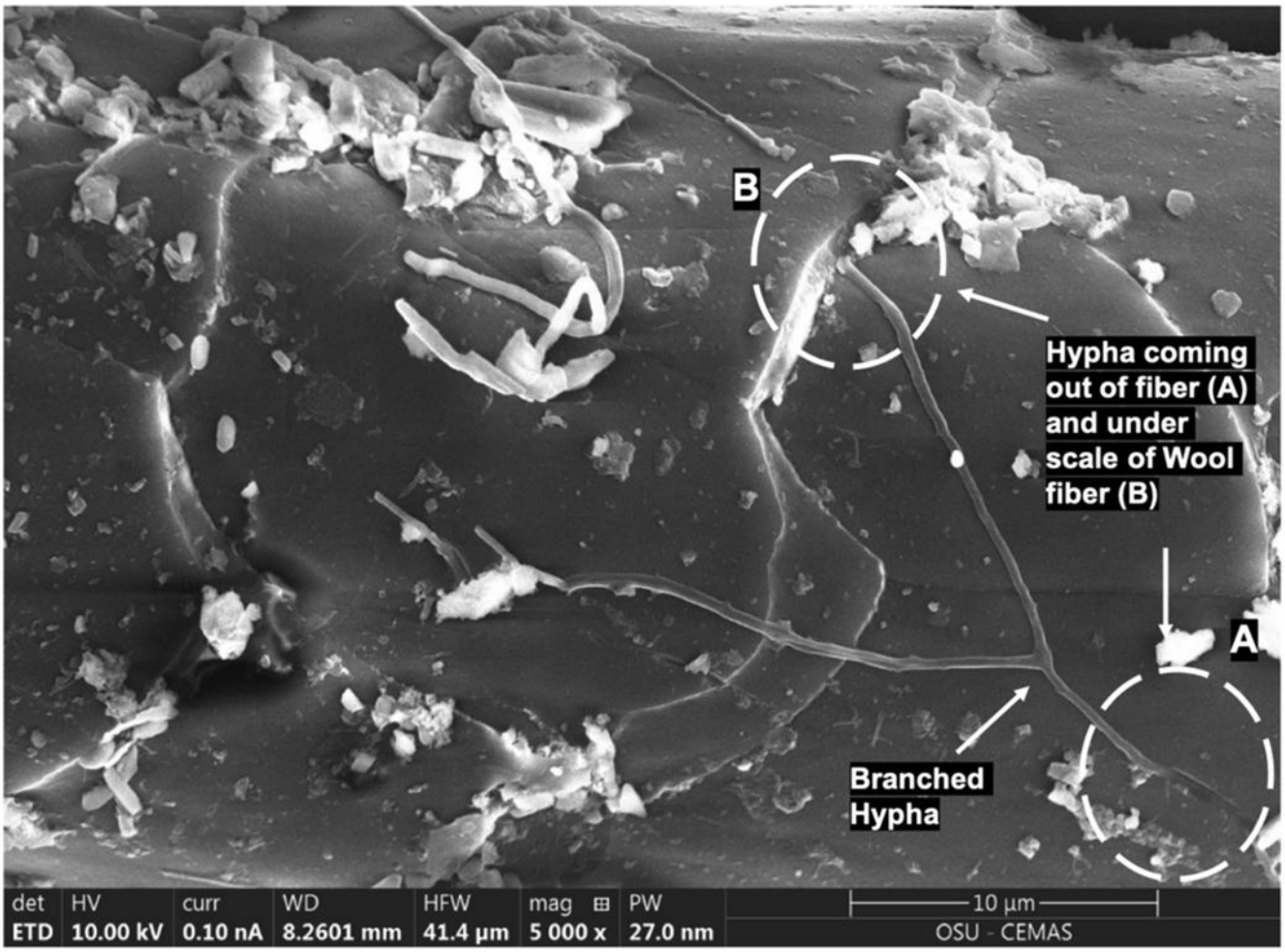

Fig. 12.

A. alternata penetrating wool carpet fiber. Carpet embedded with autoclaved dust and inoculated with *A. alternata*. Incubated for 2 weeks at 25 °C and 100% RH.

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Table 1

Carpet sample information per 5 cm by 5 cm coupon.

Sample	Pile Height (mm)	Mass of Fibers (g)	Fiber Material	Pile Type	Backing Material	New/In-Home Extraction
Olefin	**60**	**1.34**	**Olefin**	**Looped**	**Synthetic Jute**	**New**
Wool	**60**	**0.98**	**Wool**	**Looped**	**Synthetic Jute**	**New**
Nylon	**130**	**2.65**	**Nylon**	**Cut Plush**	**Felt**	**New**
Site 1	**120**	**2.62**	**Nylon**	**Cut Plush**	**Synthetic Jute**	**In-Home**
Site 2	**180**	**0.85**	**Nylon**	**Cut Plush**	**Synthetic Jute**	**In-Home**
Site 3	**100**	**1.46**	**Nylon**	**Cut Plush**	**Synthetic Jute**	**In-Home**

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Table 2

Correlatation analysis of qPCR concentrations versus ERH conditions. Average qPCR measurements from each of the 3 sites at each of the 5 ERH conditions were used to calculate the Spearman Rank Correlation Coefficient.

Sample Method	Spearman Rank Correlation Coefficient	p-value
Fiber-Dust	**0.88**	**< 0.0001**
Dust Only	**0.92**	**< 0.0001**
Vacuumed Dust	**0.87**	**< 0.0001**

Supplementary Material

Site ID	1	2	3
Weather	Rainy/Cloudy	Sunny/Warm	Sunny/Cold
Outdoor Temperature	24°C	18°C	7°C
Outdoor Relative Humidity	85%	37%	80%
Indoor Temperature	24°C	15°C	19°C
Indoor Relative Humidity	50%	42%	53%
Water Damage or Mold Growth	No	No	No
How often do you vacuum carpet?	Every Other Week	Every Other Week	Every Week
Approximate age of carpet?	11+ Years	20 Years	14 Years

Table S1: ERH Sample Site Data

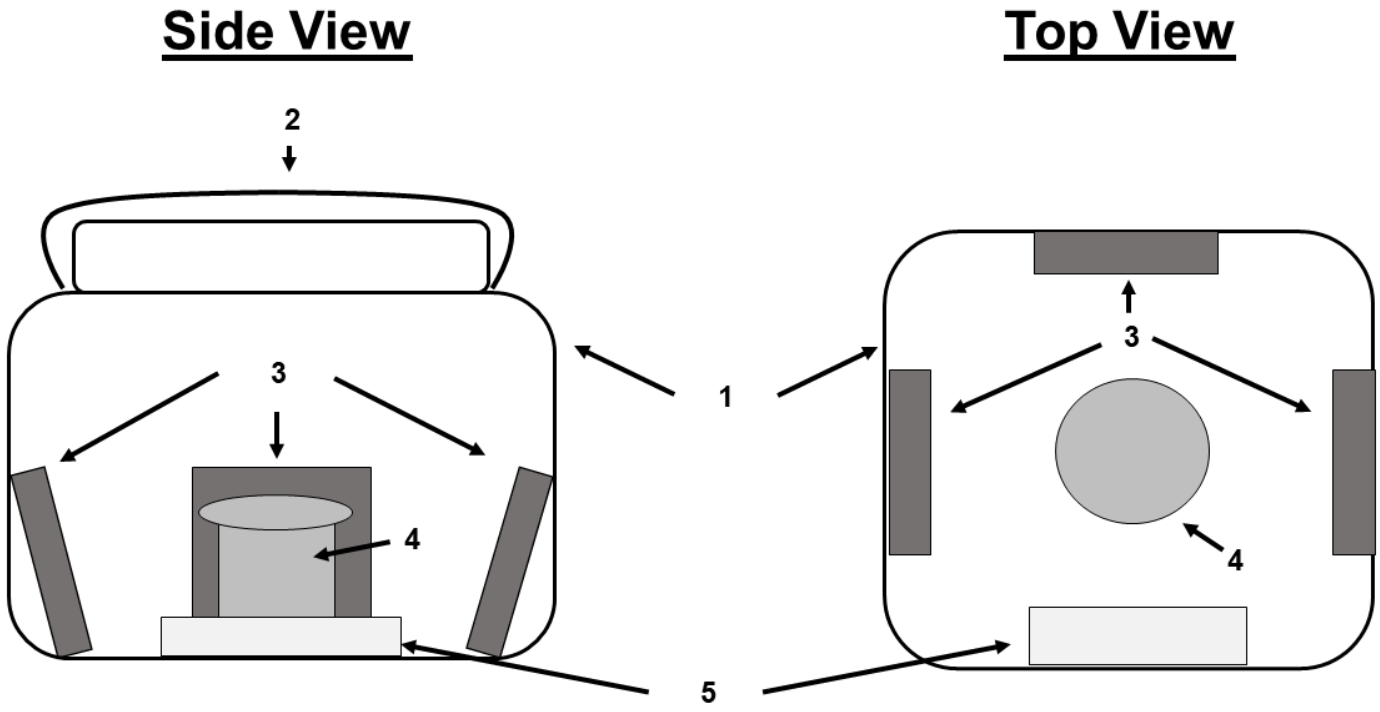

Figure S1: Schematic of Incubation Chambers. 2 L glass jars (1) were covered with parafilm (2) to keep moisture inside the chamber and allow CO_2 to escape. 5cm x 5cm carpet coupons were placed on side walls of glass jar, three per jar

(3). A beaker was placed in the center of the jar filled with salt solution to control ERH (4) and recorded using an ONSET HOBO logger (5).

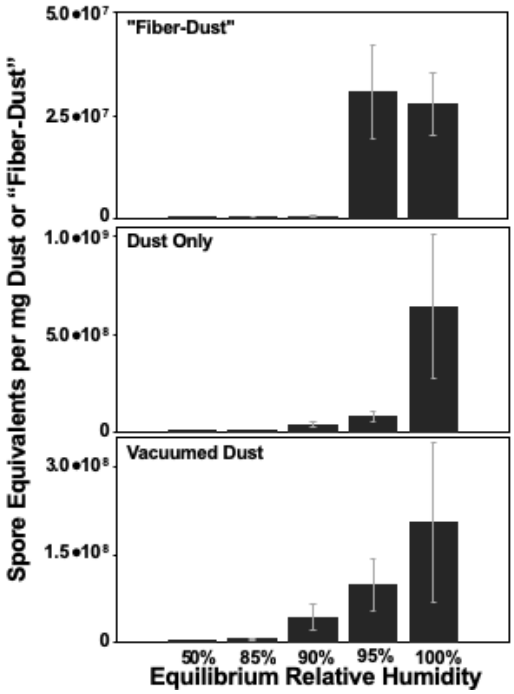

Figure S2: Average qPCR values and variation of three sites used in ERH experiment for all sampling methods ("Fiber-Dust", Dust Only, and Vacuumed Dust)

Sample	Efficiency (%)	SD (%)
50 mg Wool	100	18
50 mg Olefin	55	9
50 mg Nylon	98	17

Table S2: qPCR Carpet Fiber DNA Extraction Efficiency

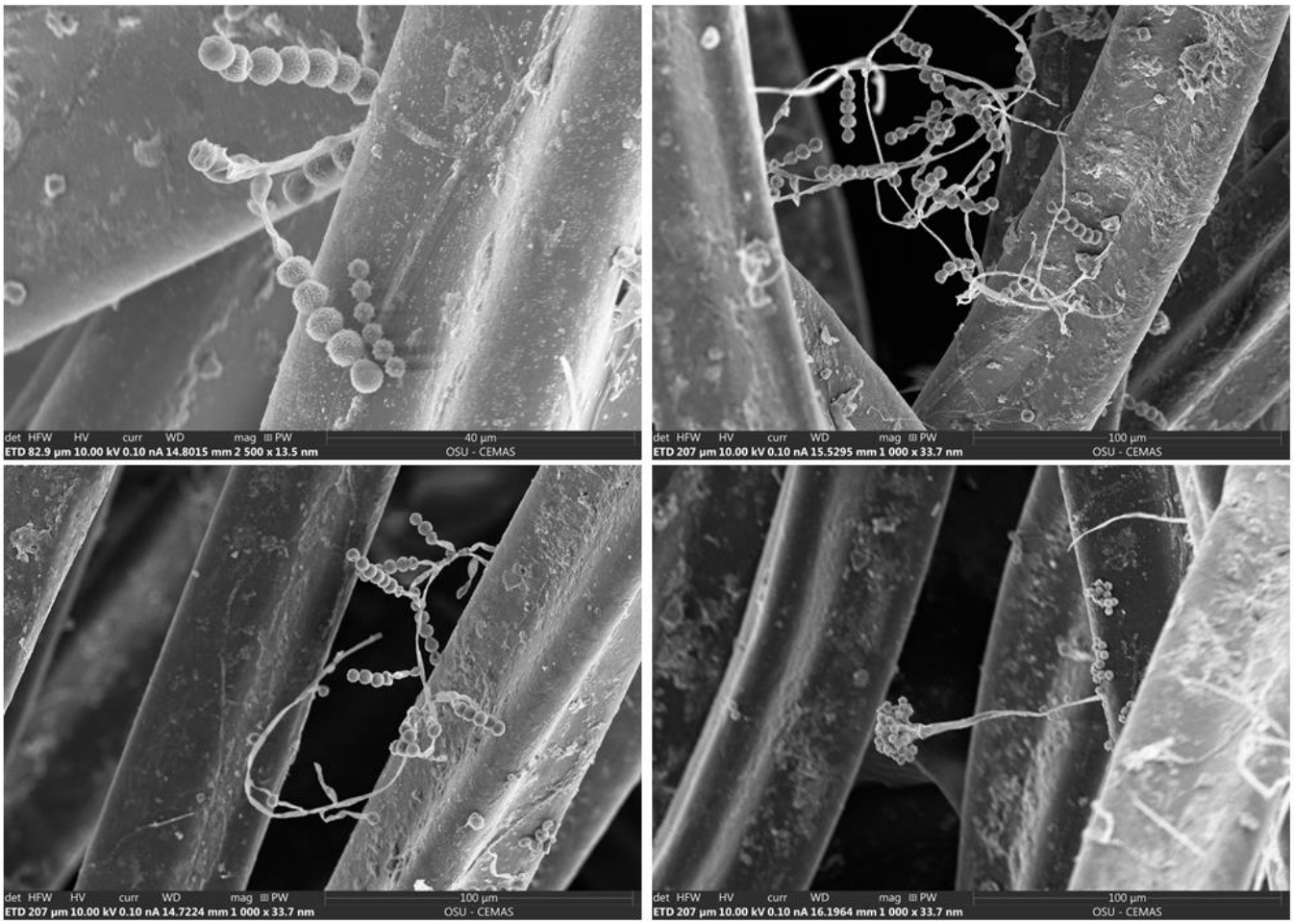

Figure S3: SEM images of fungal growth on carpet fibers. All images are from Site 2 incubated at 25°C and 95%RH for 2 weeks.

100% ERH for 2 Weeks								
Site 1			Site 2			Site 3		
Species	Quantity (Spore Equivalents per mg Dust)	% of Total Fungi Present	Species	Quantity (Spore Equivalents per mg Dust)	% of Total Fungi Present	Species	Quantity (Spore Equivalents per mg Dust)	% of Total Fungi Present
Aspergillus sydowii	62818	17.02	*Penicillium chrysogenum*	7180436	21.58	*Alternaria soliaridae*	999503	20.67
Penicillium chrysogenum	17531	4.75	*Cladosporium sphaerospermum*	5075399	15.25	*Epicoccum nigrum*	213712	4.42
Epicoccum nigrum	10482	2.84	*Acremonium charticola*	3115172	9.36	*Aspergillus austroafricanus*	179348	3.71
Aspergillus pseudodeflectus	10294	2.79	*Aspergillus unguis*	1984951	5.97	*Vishniacozyma carnescens*	148475	3.07
Cladosporium sphaerospermum	7599	2.06	*Cladosporium halotolerans*	1416308	4.26	*Phialemonium atrogriseum*	73528	1.52
Alternaria alternata	7096	1.92	*Sterigmatomyces halophilus*	1370393	4.12	*Nothophoma anigozanthi*	66983	1.39

Cladosporium delicatulum	5342	1.45	*Gibberella intricans*	1271499	3.82	*Xanthocarpia erichansenii*	65128	1.35
Acremonium alternatum	3561	0.96	*Aspergillus austroafricanus*	688728	2.07	*Nigrospora oryzae*	59237	1.22
Aspergillus melleus	2883	0.78	*Verticillium dahliae*	611026	1.84	*Scedosporium boydii*	54110	1.12
Chalastospora ellipsoidea	2405	0.65	*Aspergillus sydowii*	533323	1.60	*Aspergillus pseudodeflectus*	40473	0.84
Alternaria infectoria	2372	0.64	*Epicoccum nigrum*	430897	1.29	*Alternaria chlamydospora*	28364	0.59
Gymnascella confluens	2110	0.57	*Aspergillus pseudodeflectus*	413237	1.24	*Pringsheimia smilacis*	25855	0.53
Penicillium citrinum	1263	0.34	*Cladosporium delicatulum*	218980	0.66	*Rhodotorula graminis*	20291	0.42
Alternaria chlamydospora	1209	0.33	*Nothophoma anigozanthi*	218980	0.66	*Wallemia mellicola*	19964	0.41
Mycosphaerella tassiana	954	0.26	*Toxicocladosporium irritans*	194257	0.58	*Ochrolechia frigida*	18000	0.37
Stemphylium herbarum	947	0.26	*Cladosporium langeronii*	183661	0.55	*Penicillium bialowiezense*	17891	0.37
Chaetomium grande	927	0.25	*Engyodontium album*	173065	0.52	*Sterigmatomyces halophilus*	16800	0.35
Aspergillus austroafricanus	914	0.25	*Cladosporium dominicanum*	166001	0.50	*Xanthocarpia crenulatella*	15491	0.32
Cladosporium halotolerans	672	0.18	*Acremonium alternatum*	155405	0.47	*Byssochlamys spectabilis*	15164	0.31
Aspergillus penicillioides	611	0.17	*Sistotrema raduloides*	137746	0.41	*Cryptococcus victoriae*	15055	0.31

Table S3: Illumina ITS sequence data for all ERH sample sites. Table shows the 20 most abundant fungal species in each site from vacuumed dust samples after 2 weeks at 100% ERH and 25°C.

Original Dust (Before Incubations)								
Site 1			Site 2			Site 3		
Species	Quantity (Spore Equivalents per mg Dust)	% of Total Fungi Present	Species	Quantity (Spore Equivalents per mg Dust)	% of Total Fungi Present	Species	Quantity (Spore Equivalents per mg Dust)	% of Total Fungi Present
Epicoccum nigrum	5242	67.96	*Gibberella intricans*	9958	21.74	*Epicoccum nigrum*	5660	37.09
Mycosphaerella tassiana	306	3.97	*Epicoccum nigrum*	5105	11.15	*Cystofilobasidium macerans*	1410	9.24
Cladosporium delicatulum	185	2.40	*Mycoarthris corallina*	3129	6.83	*Nigrospora oryzae*	689	4.51
Paraconiothyrium brasiliense	155	2.00	*Epicoccum plurivorum*	1924	4.20	*Vishniacozyma carnescens*	599	3.93
Neoascochyta desmazieri	102	1.33	*Cladosporium delicatulum*	1479	3.23	*Cladosporium delicatulum*	446	2.92
Cryptococcus victoriae	88	1.14	*Neoascochyta desmazieri*	1091	2.38	*Paraconiothyrium brasiliense*	402	2.63
Filobasidium stepposum	59	0.77	*Articulospora proliferata*	817	1.78	*Xanthocarpia erichansenii*	364	2.39

Holtermanniella takashimae	**44**	**0.57**	***Nothophoma anigozanthi***	**814**	**1.78**	***Pyrenochaeta keratinophila***	**272**	**1.78**
Pyrenochaetopsis pratorum	**41**	**0.53**	***Cystofilobasidium capitatum***	**775**	**1.69**	***Pyrenochaetopsis leptospora***	**169**	**1.11**
Vishniacozyma carnescens	**37**	**0.48**	***Pyrenochaetopsis pratorum***	**624**	**1.36**	***Neoascochyta desmazieri***	**141**	**0.92**
Wallemia mellicola	**35**	**0.45**	***Ascochyta manawaorae***	**470**	**1.03**	***Xanthocarpia crenulatella***	**114**	**0.75**
Cystofilobasidium macerans	**34**	**0.44**	***Devriesia pseudoamericana***	**445**	**0.97**	***Coniosporium apollinis***	**108**	**0.71**
Monographella cucumerina	**32**	**0.42**	***Mycosphaerella ellipsoidea***	**434**	**0.95**	***Nothophoma anigozanthi***	**102**	**0.67**
Nigrospora oryzae	**28**	**0.37**	***Cystofilobasidium macerans***	**432**	**0.94**	***Pyrenochaetopsis pratorum***	**95**	**0.62**
Cucurbitaria berberidis	**28**	**0.36**	***Fusarium oxysporum***	**421**	**0.92**	***Didymocyrtis pseudeverniae***	**92**	**0.60**
Myrothecium atrum	**27**	**0.35**	***Adisciso kaki***	**410**	**0.89**	***Neoascochyta paspali***	**82**	**0.54**
Rhodotorula graminis	**27**	**0.35**	***Cryptococcus victoriae***	**390**	**0.85**	***Rhodotorula graminis***	**75**	**0.49**
Aspergillus sydowii	**26**	**0.34**	***Aspergillus austroafricanus***	**368**	**0.80**	***Cryptococcus victoriae***	**72**	**0.47**
Gibberella intricans	**23**	**0.30**	***Phaeococcomyces nigricans***	**363**	**0.79**	***Gibberella intricans***	**69**	**0.45**
Ascochyta manawaorae	**20**	**0.25**	***Paraconiothyrium brasiliense***	**349**	**0.76**	***Monographella nivalis***	**64**	**0.42**

Table S4: Illumina ITS sequence data for all ERH sample site's original dust. Table shows the 20 most abundant fungal species in each site before any incubations

	Total Fungal					
	No Dust		**Autoclaved House Dust**		**House Dust**	
	Satterthwaite Two Sample t-test P-Value	**Pearson Correlation Coefficient**	**Satterthwaite Two Sample t-test P-Value**	**Pearson Correlation Coefficient**	**Satterthwaite Two Sample t-test P-Value**	**Pearson Correlation Coefficient**
Wool & Nylon	**0.0051**	**0.06**	**0.0004**	**0.19**	**0.0006**	**0.75**
Wool & Olefin	**0.0001**	**0.95**	**0.0006**	**0.78**	**0.0054**	**0.95**
Nylon & Olefin	**0.0661**	**0.89**	**0.0203**	**0.97**	**0.0016**	**0.30**

	Alternaria alternata					
Wool & Nylon	**0.0006**	**0.98**	**0.0023**	**0.99**	**0.0005**	**0.99**
Wool & Olefin	**0.0007**	**0.98**	**0.0018**	**0.99**	**0.1250**	**0.75**
Nylon & Olefin	**0.2588**	**0.55**	**0.0001**	**0.99**	**0.0001**	**0.99**

Table S5: Statistical Analyses for Materials and Dust Loading Samples.

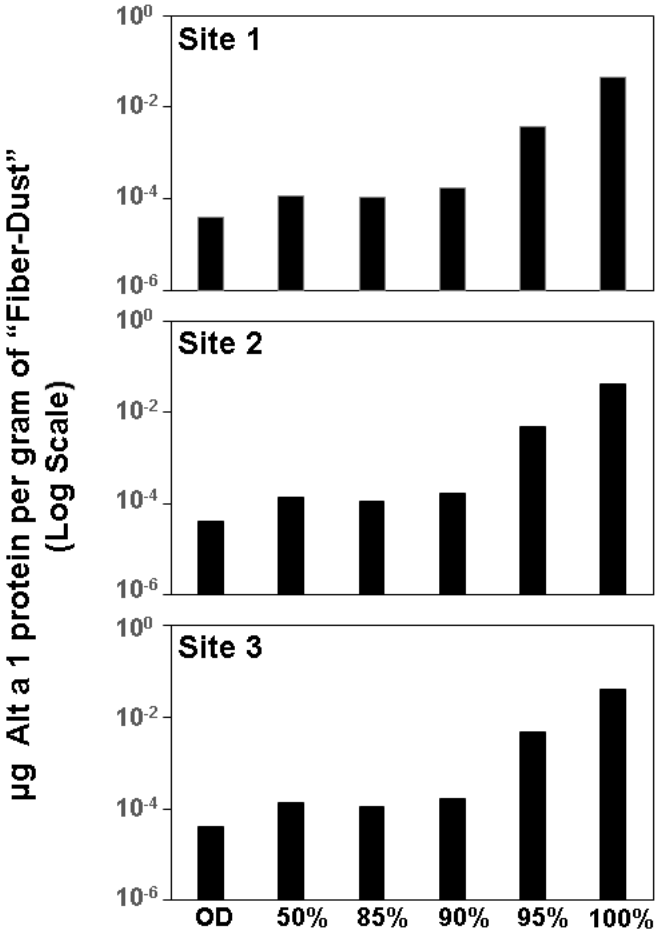

Figure S4: Alt a 1 allergen concentrations for Sites 1, 2, and 3. Samples were quantified with "Fiber-Dust" extracted from the incubated carpet coupons at each ERH condition and for each site's original dust (OD).

	Average of 3 Sites		
	Satterthwaite Two Sample t-test p-Values	**Spearman Rank Coefficient**	**Spearman Rank p-Value**
OD & 50%	**0.36**	**0.87**	**< 0.0001**
OD & 85%	**0.26**		
OD & 90%	**0.16**		
OD & 95%	**0.03**		
OD & 100%	**0.006**		

Table S6: Statistical Analyses for Alt a 1 allergen where original dust (OD) was compared to all ERH conditions using the average Alt a 1 allergen quantities from Sites 1, 2, and 3. Logarithmic transformed Alt a 1 concentration values were used for the Satterthwaite Two Sample t-test to reflect the values and scale in Figure 10.

Site	Total *Alternaria sp.* present (Spore equivalents per mg dust)	% *Alternaria sp.* in sample	Alt a 1 Concentration (µg Alt a 1 per g dust)
1	11584	3.14	0.05
2	67107	0.20	0.04
3	1036267	21.43	1.07

Table S7: Total concentration of Alternaria sp. based on Illumina ITS sequencing/qPCR quantification and corresponding Alt a 1 concentration. Samples were incubated for 2 weeks at 100% ERH and 25°C.